SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

SIR BENJAMIN SIMPSON, M.D., K.C.I.E.,
SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

PART V.

1890.

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3.—A List of Laccadive Plants.—*Surgeon David Prain, M.A., M.B.* *(Page 47.)*

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On Milk as a Medium for Choleraic Comma-Bacilli.

BY

SURGEON-MAJOR D. D. CUNNINGHAM, M.B., F.R.S.,

BENGAL MEDICAL SERVICE.

In a paper which was published in the last number of this periodical, an account was given of the results of a series of experiments on the behaviour of Choleraic Comma-Bacilli when introduced into water and soil of various qualities, and it was pointed out that these appeared to demonstrate that the Bacilli were very feeble organisms when exposed to a struggle for existence in these media. As a supplement to these experiments another series was subsequently tried in regard to the phenomena presenting themselves where milk was employed as a medium, and these gave results which appear to be sufficiently remarkable and sufficiently opposed to current beliefs to merit detailed description.

A special interest attaches to the behaviour of Comma-Bacilli in milk, as the latter is so often, and frequently on very insufficient evidence, regarded as the exciting cause of cases of cholera, and as it has also been affirmed to be a specially favourable medium for the development of the Bacilli. For, in the first place, if milk really does play an important part as a vehicle in the propagation of cholera, and were, at the same time, a favourable medium for the growth and multiplication of Comma-Bacilli, the evidence would, in so far, at all events, be in favour of the views of those who regard the Bacilli as the specific cause of the disease. But, apart from the question of causal relation, there can be no question as to the frequency of the association of the Bacilli with the disease as characteristic constituents of the alvine discharges—a frequency which is so great that the association is generally regarded as of diagnostic value—so that, at all events, were milk a really favourable medium for the Bacilli, we should have an invaluable aid towards determining whether in any case a suspected sample of the fluid had really been contaminated with choleraic materials. In fact, for all those believing in the constant specific association of Comma-Bacilli with choleraic discharges, and regarding the latter as the material normally containing the poison, no assertion of the agency of milk as a vehicle could be regarded as of any conclusive value without the demonstration of the presence of Comma-Bacilli in it—a demonstration which could, of course, be readily afforded by means of suitable cultivations. Moreover, independently of any questions specially relating to cholera, or any individual disease, we find milk, of the very impure quality ordinarily in use in India, so frequently regarded as a cause of disease that it appears to be very desirable to obtain some definite data regarding its normal behaviour in relation to schizomycete organisms generally, and specially in regard to its relation to any, such as Comma-Bacilli, to which definite pathogenic properties have been ascribed.

In dealing with any subject of this nature it cannot be too clearly borne in mind that we must distinguish between the potential capacities which any material may possess as a medium under special conditions, such as those of sterilisation, from those which it possesses under ordinary circumstances and when already populated by schizomycete organisms. From the point of view of practical hygiene it is of comparatively little moment what the properties of milk may be when in an exceptional condition, such as that of sterilisation, and it is its properties in the condition in which it ordinarily comes into use which alone merit serious consideration. This, however, would seem, to a great extent, to have been overlooked, and the results of experiments on sterilised milk to have been regarded as affording sufficient grounds for statements referring to the fluid generally.

The experiments, of which it is proposed to give an account here, were accordingly, not confined to an investigation of the phenomena presenting themselves in sterilised specimens of milk, but dealt with the fluid as ordinarily supplied for use in the European houses and native bazaars of Calcutta, with specimens procured under special precautions against contamination, with specimens which had been boiled, and with others which had been really sterilised. The results have been included in tabular form in order, so far as possible, to avoid tedious repetition of details in the text, which can thus be confined to the description of the general phenomena presenting themselves in each group of experiments—

TABLE I.—*Changes occurring in Unboiled Specimens of Milk from various sources and under various conditions.*

Number of specimen.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
I	Mar. 1st.	House supply.	Taken in a sterilised tube. Evenly fluid. Faintly alkaline.	1. Tube in the open laboratory.	March 2nd. Odourless; strongly and permanently acid; coagulated and separated into coagulum and abundant pale, straw coloured fluid.
II	Mar. 8th.	„	Taken in a sterilised tube. Neutral.	1. Tube in the incubator at 37°C.	March 9th. Strongly acid; separated into firm, white coagulum and clear, pale, straw-coloured fluid.

Number of specimen.	Date at which procured.	Source.	General condition wheih obtained.	Nature and treatment of individual samples.	Subsequent condition.
III	Mar. 10th.	House supply.	As in the previous specimen.	1. Tube in the incubator at 37°C.	March 11th. Strongly acid; fully coagulated and separated into coagulum and fluid.
IV	Mar. 12th.	„ .	As in the previous specimen.	As in the previous specimen.	March 13th. Highly acid and fully coagulated.
V	Mar. 15th.	Bazaar .	As in the previous specimen.	1. Tube in the incubator at 37°C.	March 16th. Strongly acid; odourless; fully coagulated and separated into coagulum and fluid.
				2. Tube in the open laboratory.	March 16th. Strongly acid; coagulated; separation of coagulum and fluid beginning.
VI	May 9th.	Zoological Garden.	Obtained direct in a sterilised beaker from a cow, the udder and teats of the animal and the hands of the milkman having been washed with solution of corrosive sublimate and subsequently with spirit. Reaction neutral.	1. Tube in the incubator at 37°C.	May 10th. Reaction transitorily feebly acid, becoming neutral on drying; not coagulated. May 11th. Very faintly acid; not coagulated. May 13th. Faintly acid; fluid, but coagulating on being boiled.
VII	May 21st.	House supply.	In a sterilised tube. Reaction transitorily faintly acid; neutral on drying.	1. Tube in the incubator at 37°C.	May 22nd. Reaction acid; fully coagulated and separated.
VIII	May 23rd.	House .	In a sterilised tube. Reaction transitorily neutral; faintly alkaline on drying.	1. Tube in the incubator at 37°C.	May 24th. Reaction acid; fully coagulated and separated.
IX	May 25th.	„ .	As in the previous specimen.	As in the previous specimen.	May 26th. Reaction acid, becoming fainter on drying; softly coagulated, but not separated until boiled.
X	May 27th.	Bazaar .	As in the previous specimen.	As in the previous specimen.	May 28th. Reaction at first strongly acid, becoming feeble on drying; completely coagulated and separated.
XI	May 29th.	„ .	Obtained in a sterilised flask.	1. Cream skimmed from the top; in a sterilised tube in the incubator at 37°C. 2. Skimmed milk; in a sterilised tube in the incubator at 37°C.	May 30th. Fully coagulated and separated.

Number of specimen.	Date at which procured.	Source.	General condition which obtained.	Nature and treatment of individual samples.	Subsequent condition.
XII	May 31st.	Bazaar .	The milk siphoned into distinct samples derived from the upper and lower strata.	1. Tube of the top milk in the incubator at 37°C.	June 1st. Reaction acid; fully coagulated and separated.
				2. Tube of the bottom milk in the incubator at 37°C.	June 1st. Reaction acid, but not so strongly so as that of the previous sample; fully coagulated and separated.
XIII	June 7th.	„ .	Milk treated as in the previous specimen. Reaction neutral.	1. Tube of the top milk in the incubator at 37°C. 2. Tube of the bottom milk in the incubator at 37°C.	June 8th. Acid; fully coagulated and separated.
XIV	July 15th.	„ .	Milk treated as above. Reaction faintly alkaline on drying.	1. Tube of bottom milk in incubator at 37°C.	July 16th. In soft coagulum, but not separated. July 17th Fully coagulated and separated; reaction strongly acid.
XV	July 25th.	„ .	Milk treated as above. Reaction neutral.	1. Tube as in the previous case.	July 26th. Reaction acid; in soft coagulum, but not separated.
XVI	July 29th.	„ .	Treated as above. Reaction neutral.	1. Tube as in the previous case.	July 30th. Acid; in soft coagulum, afterwards undergoing separation.
XVII	Aug. 3rd.	„ .	Treated as above. Reaction neutral.	1. Tube as in the previous case.	August 4th. Not coagulated until it was boiled, when coagulation and separation occurred at once.
XVIII	Aug. 15th.	„ .	Treated as above. Reaction faintly alkaline.	1. Tube as in the previous case.	August 15th. Reaction strongly acid; coagulated and partly separated, with a little froth above.

This table shows the changes which specimens of milk of various qualities normally tend to undergo if left without any special treatment. When first obtained, the milk as a rule was neutral in reaction, in some cases faintly alkaline on drying, and in one transitorily faintly acid. After an interval of 24 hours the reaction in all ordinary samples of milk is acid and in most cases strongly so, and, in association with this change in reaction, coagulation has occurred, in most cases fully developed and with separation into coagula and fluid, but in some not so far advanced and with an absence or incompleteness of separation. In rare instances a certain amount of frothing is present, but as a rule the surface of the fluid is merely covered with a layer of coagulated cream. The occurrence of coagulation appears to be influenced by temperature, as in some instances in which corresponding samples of milk were set simultaneously in the open Laboratory and the Incubator at a temperature of 37°C., complete coagulation occurred more rapidly in the incubated specimens than in those exposed to the ordinary atmospheric temperature, which, as a rule, was considerably lower than that of the incubator, and in no instance was constantly at such a high level during the entire period of 24 hours. In only one case was there neither permanent acidity nor coagulation after the specimen had been under observation for a period of 24 hours, and in this the milk had been obtained under very special precautions. The specimen is that which is numbered VI in the Table and was drawn direct into a sterilised beaker from which the plug of cotton wool had just been withdrawn and at once transferred thence to a sterilised and plugged flask, and finally to a sterilised tube. The udder and teats of the cow and the hands of the milkman had both been freshly washed, first with a 5% solution of corrosive sublimate and then with spirit, immediately before the milk was withdrawn. In spite of being kept constantly in the incubator, spontaneous coagulation had not occurred up to a period of 96 hours afterwards, and only set in on boiling. The reaction too was quite exceptional, as it was only transitorily acid after the lapse of 24 hours, and only faintly acid permanently after 96 hours. The result clearly shows that the development of acidity and of coagulation is determined, principally at all events, by contamination of the fluid during drawing and subsequent preservation.

TABLE *II.—Results of Plate Cultivations of Unboiled Specimens of Milk from various sources.*

Number of specimen.	Date at which procured.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
I	Jan. 2nd.	Bazaar	...	Plate. Inoculated with 0·083 C. C. Set in incubator at 37°C.	Jan. 2nd.	January 3rd. Crowded with concrete colonies in the proportion of about 100,000 per C. C. The colonies were both superficial and interstitial. In addition to these there were several bluish, diffuse patches of growth, and two of a bright greenish yellow tint. The schizomycete forms present included two kinds of Micrococci; a small Baccillus, the individual rods with rounded ends and often united in pairs; a slender straight Bacillus; and a limited number of larger rods, apparently of *Bacillus subtilis,* which occurred among the other forms and must apparently have been due to a thin, diffuse, superficial growth contaminating the specimens derived from the distinct colonies. Smell of the cultivation mouldy, non-choleraic.
II	Jan., 7th.	House-supply.	...	Plate. Treated as in the previous case.	Jan. 7th.	January 8th. A growth of colonies, similar to those in the previous case, in the proportion of 20,000 per C. C.

Number of specimen.	Date at which procured.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
III	Jan. 9th.	House-supply.	...	Plate. Treated as in the previous specimen.	Jan., 9th.	January 10th. A growth of colonies in the proportion of 48,150 per C. C. Almost all of them appeared to consist of the short, oval, often paired Bacilli described in the account of the cultivation of specimen I. As in that case scattered rods of larger size and apparently due to diffuse growth of *Bacillus subtilis* were also present.
IV	Jan. 16th.	„ .	Neutral .	„ .	Jan. 16th.	January 17th. Crowded with concrete colonies in the proportion of 132,000 per C. C. Also a thin, diffuse, superficial growth visible at one point. The defined colonies consisted of the short oval Bacilli and the smaller form of Micrococcus occurring in the cultivation of specimen I. The diffuse growth consisted of *Bacillus subtilis*.
V	Mar. 1st.	„ .	Faintly alkaline.	„ .	Mar. 1st.	March 2nd. Small colonies in the proportion of 80,000 per C. C. One definite patch and apparently a very thin, generally diffused film of *Bacillus subtilis*. The small colonies consisted of the common short oval Bacilli occurring in the previous cultivations and of minute straight Bacilli and large Micrococci similar to those present in the cultivation of specimen I.
				„ .	Mar. 4th.	March 5th. Crowded with minute colonies of short oval Bacilli and large Micrococcus.
VI	Mar. 8th.	„ .	Neutral .	„ .	Mar. 8th.	March 9th. Colonies present in the proportion of 103,950 per C. C. These almost entirely consisted of the large Micrococcus occurring in several of the previous cultivations. As in other cases, a very limited, superficial, diffuse growth of *Bacillus subtilis* appeared to be present, the stratum, however, being too thin to be recognisable.
				„ .	Mar. 9th.	March 10th. Crowded with minute colonies of the large Micrococcus and the oval Bacillus occurring in previous cultivations.
VII	Mar. 10th.	„ .	„ .	„ .	Mar. 10th.	March 11th. Colonies present in the proportion of 51,000 per C. C. The greater number of these consisted of the common lacteal oval Bacilli. One large foliated patch of the large Micrococcus.
VIII	Mar. 12th.	„ .	„ .	„ .	Mar. 12th.	March 13th. Crowded with colonies in the proportion of 340,000 per C. C. These consisted of *Bacillus subtilis*, the common lacteal oval Bacilli, large Micrococcus and slender straight Bacilli. A certain number also were apparently composed of some form of yeasty growth of cells of some higher fungal species.
IX	May 9th.	Zoological Garden.	„ .	Plate. Inoculated with 0·058 C. C. Set in incubator at 37°C.	May 9th.	May 10th. Colonies of the common, large, lacteal Micrococcus in the proportion of 25,245 per C. C.

Number of specimen.	Date at which procured.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
				Plate. Treated as before.	May 11th.	May 13th. Absolutely crowded with colonies of the common, large, lacteal Micrococcus.
X	May 21st.	House-supply.	Faintly acid, becoming neutral on drying.	Plate. Treated as before.	May 21st.	May 22nd. Absolutely crowded with colonies of the common, short, oval, lacteal Bacillus.
XI	May 23rd.	„ .	Neutral, becoming faintly alkaline on drying.	„ .	May 23rd.	May 24th. Relatively very few colonies, the proportion being only 3,400 per C. C. They consisted of the common lacteal oval Bacilli and large Micrococcus. A very thin, diffuse growth of *Bacillus subtilis* appeared also to be present.
				„ .	May 24th.	May 25th. Crowded with colonies of the common lacteal oval Bacilli and large Micrococci.
XII	May 25th.	„ .	„ .	„ .	May 25th.	May 26th. Crowded with colonies of the common lacteal oval, and small straight Bacilli, and large Micrococcus. A thin, diffuse growth of *Bacillus subtilis* appeared also to be present.
XIII	May 27th.	Bazaar	„ .	„ .	May 27th.	May 28th. Number of colonies very small, only giving a proportion of 8,670 per C. C. They appeared to consist entirely of the common large, lacteal Micrococcus. There was also, seemingly, a very thin, diffuse growth of *Bacillus subtilis* present.
XIV	May 29th.	„ .	...	„ .	May 29th.	May 30th. Numerous colonies were present. These consisted of large Micrococci and common lacteal oval Bacilli, which, where situated at the lower surface of the Agar-Agar, had in many cases elevated it into bubbles, due to gaseous evolution.
XV	May 31st.	„ .	...	Plate. Inoculated with 0·058 C. C. Siphoned off from bottom of the milk.	May 31st.	June 1st. } Both plates alike. Containing numerous colonies of the common lacteal Schizomycetes.
				Plate. Similarly inoculated from top of milk.	„ .	„
XVI	June 7th.	„ .	Neutral .	Plate. Inoculated with 0·058 C. C. of milk from the top.	June 7th.	June 8th. Numerous colonies of the common lacteal Schizomycetes.
				Plate. Inoculated with 0·058 C. C. from the bottom.	„ .	June 8th. Colonies not nearly so abundant as in the previous cultivation, but of the same nature.

Number of specimen.	Date at which procured.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
XVII	July 15th.	Bazaar	Faintly alkaline on drying.	Plate. Inoculated with 0·03 C. C. from the bottom of the milk.	July 15th.	July 16th. Crowded with colonies of common lacteal Schizomycetes in the proportion of 340,965 per C. C. Small straight Bacilli and short oval ones the most prevalent forms.
XVIII	July 25th.	„ .	Neutral .	Plate. Inoculated as above with 0·03 C. C.	„ .	July 26th. Smell mouldy. Abundant colonies of typical lacteal Micrococcus, oval and small straight Bacilli.
XIX	July 29th.	„ .	„	Plate. Inoculated as above.	July 29th.	July 30th. Numerous colonies in the proportion of 51,172 per C. C. Most of them consisting of the common lacteal large Micrococcus and short oval Bacilli. A few superficial bluish colonies of small, straight Bacilli also present.
XX	Aug. 15th.	„ .	Faintly alkaline.	„ .	Aug. 15th.	August 16th. Colonies present in the proportion of 39,847 per C. C. Consisting of common lacteal Micrococcus and small straight Bacilli.

This table records the results of cultivations of unboiled milk. These show that the milk in common use in Calcutta contains a varying but always very large number of schizomycete organisms. The numbers actually present in those cases where an estimate was made by numeration of colonies present within determined areas of plate-cultivations ranged from a proportion of only 3,400 per C. C. of milk to over 300,000 per C. C.

Determinations of this nature were made in regard to 14 specimens with the following results :—

Case 1	100,000	Schizomycetes	per	C. C.	of milk.
2	20,000	„	„	„	„
3	48,000	„	„	„	„
4	132,000	„	„	„	„
5	80,000	„	„	„	„
6	103,950	„	„	„	„
7	51,000	„	„	„	„
8	340,000	„	„	„	„
9	25,245	„	„	„	„
10	3,400	„	„	„	„
11	8,670	„	„	„	„
12	349,965	„	„	„	„
13	51,172	„	„	„	„
14	39 847	„	„	„	„

One noteworthy point is that the exceptional specimen of milk procured under special precautions, and which did not undergo the normal acid fermentation and coagulation, was not free from schizomycetes, nor did it even contain an exceptionally small number of them. The figures corresponding to it are those of No. 9 in the above series, and are greatly in excess of those in Nos. 10 and 11, corresponding with samples of milk in regard to which no special precautions had been taken, and which underwent the normal and rapid change in reaction and coagulation. It was, however, a pure cultivation of one only of the species of schizomycetes commonly present in milk. In cases where successive cultivations of one and the same sample of milk were made at different periods subsequent to procural there was ample evidence that, for some time, a very rapid multiplication of schizomycetes took place, the successive cultures showing progressive increase in the numbers of colonies which developed in them. A rapid multiplication of schizomycetes thus occurs during the period in which the milk is becoming acid and coagulating, but all the species normally present in common specimens of milk do not appear to be equally active in producing these changes. The exceptional sample of milk ultimately became acid in reaction, but, even after an interval of 96 hours, was only feebly so, and only coagulated on being heated, although it was at the same time crowded with specimens of one of the common lacteal organisms. As specimens of sterilised milk inoculated with what seemed to be a pure cultivation of the same organism in other cases showed coagulation within 24 hours, although with only transitory acidity, it is a little difficult to account for the great delay manifesting itself in this particnlar instance.

There was on the whole a remarkable uniformity throughout the entire series of specimens in regard to the nature of the schizomycetes which they contained. In regard to those which gave rise to definite colonies, two forms were characterised by the extreme regularity with which they made their appearance in the cultivations, one of them having occurred in 21 of the entire series of 24 cultivations and in 17 of the 20 samples of milk, and the other in 19 of the 24 cultivations and 16 of the 20 samples. The first consists of a large species of Micrococcus and was that which was alone present in the specimen of milk, in regard to which special precautions against contamination were taken at the time when it was obtained. The other was a very short Bacillus, the extremities of the rods being rounded so as to cause them to assume an oval form, and there being a great tendency for association of the rods in pairs. The species occurring next in order of frequency was another species of Bacillus distinguished from the previous one by the greater length and relative slenderness of its rods. It was present in 11 cultivations derived from 10 distinct samples of milk. A certain amount of *Bacillus subtilis* was recognised in nine cultivations, derived from as many distinct samples of milk. In only one case were definite colonies of it present in all the others it appeared to be present in the form of diffused films spread out over the surface of the plate, and often so thin as to escape notice entirely until the presence of rods contaminating specimens derived from colonies of the other schizomycetes served to reveal them. It is very probable that growths of *Bacillus subtilis* of this nature were present in other cultivations of the series than those of which it is recorded, as the results of cultivations of boiled milk, as will be shown presently, clearly demonstrate that spores of *Bacillus subtilis* are of constant occurrence in ordinary samples of milk. The schizomycetes, which may be fairly regarded as normal constituents of ordinary samples of milk in Calcutta, are thus a large Micrococcus, an oval Bacillus, a small slender Bacillus, and finally *Bacillus subtilis*.

Other forms, such as the small species of Micrococcus occurring in specimen I of Table II, and one or two other Bacilli, sometimes occur in small numbers, but their appearance is an exceptional, not a normal phenomenon. In the case of one specimen, No. VIII of Table II, cultivation, in addition to colonies of common lacteal schizomycetes, yielded others composed of dense masses of yeast-like cells. The first four photographs in the plate show the proportions of colonies present in cultivations of unboiled specimens of milk of twenty-four hours' growth. The first three are from cultivations set immediately after the milk was obtained and figure 3, when taken along with the two previous ones, shows the relatively small numbers of colonies appearing in a cultivation of the specimen of milk obtained under special precautions to avoid contamination, as compared with the numbers present in cultivations of specimens of the common milk supply of Calcutta. The fourth figure,compared with the third,shows the excessive multiplication of schizomycetes in specimens of milk when kept for any length of time. Both cultivations to which the figures correspond were obtained from the same sample of milk, but that illustrated in figure 3 was set immediately after the sample was obtained, while that of figure 4 was not set until 48 hours later. One point which came out very clearly in this series of cultivations was the relative repression, and in many cases apparent *complete* suppression of *Bacillus subtilis* in milk, which is associated with the presence of the other schizomycetes normally present in the fluid and the acid fermentation with which their presence and multiplication is associated. As will be shown in the fourth Table, spores of *Bacillus subtilis* appear to be a constant content of ordinary specimens of milk, but the development of the species in all these cultivations was inconspicuous, and in many instances no development at all seemed to occur. The repressive influence of the other forms was specially indicated by the fact that when growths of *Bacillus subtilis* did occur they were associated with the first cultivations derived from any specimen, that is, at periods when the numbers of the common lacteal schizomycetes were relatively small, as compared with what they were subsequently.

Table III.—Changes occurring in Boiled Specimens of Milk from various sources and under various conditions.

Number of specimen.	Number of corresponding specimen of unboiled milk in Table I.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
I	...	Jan. 9th.	House-supply.	...	1. Boiled in a sterilised tube and set in the incubator at 37°C.	January 10th. Remaining apparently unaltered; quite fluid.
					Again boiled, January 10th and 11th.	The specimen throughout remained seemingly unaltered, save for a faint brownish tinge which it acquired in connection with the repeated boiling. Subsequent to the 2nd boiling it had a faint acid reaction. It remained fluid for as long as it was kept in the laboratory.
II	...	Jan. 16th.	„	Reaction neutral.	1. Boiled in a sterilised tube and set in the incubator at 37°C.	January 17th. Remaining apparently unaltered; quite fluid.
					Again boiled on January 17th.	Subsequent to the second boiling it remained unaltered during a period of many weeks in which it was retained in the laboratory.
III	II	Mar. 8th,	„	Neutral .	Treated as above .	March 9th. Reaction strongly acid; fully coagulated and separated into coagulum and fluid.
IV	III	Mar. 10th.	„	„ .	„ „ .	March 11th. Remaining fluid and seemingly unaltered.
					Re-boiled on March 11th.	March 13th. Remaining unaltered.
V	IV	Mar 12th.	„	„ .	Treated as above .	March 13th. Reaction neutral quite fluid.
					Boiled again on 13th March.	March 14th. Seemingly unaltered.
VI	V	Mar. 15th.	Bazaar.	„ .	Treated as above .	March 16th. Coagulated and separated into coagulum and fluid; smell caseous; reaction acid, becoming feeble on drying.
VII	VI	May 9th.	Zoological Garden.	„ .	„ „ .	May 10th. Quite fluid; reaction neutral, becoming feebly alkaline on drying.
					Re-boiled once in the next five days and twice on the 6th day.	Remained unaltered throughout as long as it was retained in the laboratory.

Number of specimen.	Number of corresponding specimen of unboiled milk in Table I.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
VIII	...	May 9th.	Zoological Garden.	Obtained at the same time as the previous specimen, but from a different cow. The udder was not in this case specially prepared and the calf had to be allowed to suck ere the animal would let down her milk. It was drawn direct into a sterilised beaker and transferred to three sterilised flasks.	1. Boiled in a sterilised flask and set in the incubator at 37°C.	May 10th. Fully coagulated and separated. Reaction acid.
					2. Treated as above.	May 10th. Coagulated, but separation only beginning; complete separation occurring at once of boiling.
					3. „ „ .	May 10th. Result as in sample 2.
IX	VII	May 21st.	House-supply.	Re-action faintly acid, becoming neutral on drying.	1. Boiled in a sterilised flask and set in the incubator at 37°C.	May 22nd. Fully coagulated and separated; reaction acid, becoming very faintly so on drying.
					2. Treated as above.	May 22nd. In soft coagulum, which on boiling underwent complete separation; reaction acid, becoming very faint on drying.
					3. Boiled in a sterilised tube and set in the incubator at 37°C.	May 22nd. Fully coagulated and separated; reaction acid faintly so on drying.
X	VIII	May 23rd.	„	Neutral, becoming faintly alkaline on drying.	1. In sterilised flask at 37°C.	May 24th. In soft coagulum, undergoing separation on boiling.
					2. „ .	
					3. In sterilised tube at 37°C.	May 24th. Fully coagulated and separated; reaction acid, faintly so on drying.
XI	IX	May 25th.	„	Reaction neutral, faintly alkaline on drying.	1. Boiled in a sterilised flask at 8 A.M., and 1 P.M. During the interval and subsequently in incubator at 37°C.	May 26th. Undergoing full coagulation and separation on being anew boiled.
					2. Treated as above.	May 26th. Results as above.

Number of specimen.	Number of corresponding specimen of unboiled milk in Table I.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
					3. Boiled in a sterilised tube and set in the incubator at 37°C.	May 26th. Fully coagulated and separated.
					4. Residuum in the flask, clean but non-sterilised, in which the specimen was procured. Twice boiled like 1 and 2, and left in the open laboratory.	May 26th. Did not undergo coagulation on being boiled anew.
XII	X	May 27th.	Bazaar.	Reaction neutral; faintly alkaline on drying.	1. Boiled in a sterilised flask and set in the open laboratory.	May 28th. Not visibly coagulated until reboiled, when coagulation and separation occurred.
					2. Boiled in a sterilised tube and set in the incubator at 37°C.	May 28th. Fully coagulated and separated; reaction acid, becoming neutral on drying.
XIII	XI	May 29th.	„	Obtained in a sterilised flask	1. Boiled in a sterilised flask and set in the laboratory.	May 30th. In soft, loose coagulum. Full coagulation and separation taking place on boiling.
					2. A portion, after the cream had been allowed to rise and partially skimmed off, treated as above.	
					3. Cream: boiled in a sterilised tube and set in the incubator at 37°C.	May 30th. Fully coagulated and separated.
					4. Skimmed milk: treated as 3.	
					5. Residuum in the flask remaining after the milk for the previous specimens had been removed; boiled and set in open laboratory.	May 30th. Remaining unaltered and not coagulating on being boiled anew.
XIV	XII	May 31st.	„	Siphoned into separate samples from the top and bottom.	1. Top-sample; boiled in a sterilised tube and set in the incubator at 37°C.	June 1st. Fully coagulated and separated: reaction acid.

Number of specimen.	Number of corresponding specimen of unboiled milk in Table I.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
					2. Bottom-sample; boiled in a sterilised tube and set in the incubator at 37°C.	June 1st. Quite fluid; non-coagulant on boiling: reaction feebly alkaline on drying.
					3. Bottom-sample; boiled in a sterilised flask and set in the open laboratory.	June 1st. Just as sample 2.
					4. Nature and treatment as in 3.	
					5. Residuum remaining after the milk for the previous samples had been drawn off: treated as 3 & 4.	June 1st Softly coagulated and undergoing complete coagulation and separation on boiling.
XV	XIII	June 7th.	"	Reaction neutral. Milk from the bottom siphoned off into 34 sterilised tubes, and milk from the surface into two sterilised tubes.	1. Thirty-three of the sterilised tubes of top-milk boiled at 10 A.M. and again at 6 A M. Set in the incubator in the interval and after the second boiling.	June 8th. All alike quite fluid and neutral: non-coagulant on being boiled anew. June 9th. Milk still fluid; contents of one tube coagulating on being anew boiled: all boiled again on the 9th and kept in a boiling water-bath for some hours both on the 11th and 12th. One more tube coagulated on the 13th. All the rest, which have not yet been employed in inoculation experiments, remain fluid and unaltered save slight browning up to the present date, September 6th.
					2. Residuum after removal of the milk for the tubes: boiled for a long time and left in the open laboratory.	June 8th. Coagulating and separating on boiling.
XVI	...	July 20th.	"	Neutral .	1. Milk from the bottom; boiled in a sterilised tube and set in the incubator at 37°C.	July 21st. Quite fluid: neutral.
XVII	XV	July 25th.	"	" .	1. Milk from the bottom; boiled in a sterilised tube; in the incubator at 37°C.	July 26th. Quite fluid.

Number of specimen.	Number of corresponding specimen of unboiled milk in Table I.	Date at which procured.	Source.	General condition when obtained.	Nature and treatment of individual samples.	Subsequent condition.
XVIII	XVI	July 29th.	Bazaar.	Neutral	1. Milk from the bottom; boiled in a sterilised tube; in the incubator at 37°C.	July 30th. Faintly alkaline; quite fluid.
						July 31st. Faintly alkaline; in soft loose coagulum with a little supernatant fluid.
XIX	XVII	Aug. 3rd.	„	„	1. Nature and treatment as above.	August 4th. Quite fluid and seemingly unaltered.
						August 8th Reaction faintly acid, almost neutral on drying; loosely coagulated and partially separated into coagulum and fluid.
XX	XVIII	Aug. 15th.	„	Faintly alkaline.	1. Nature and treatment as above.	August 16th. Quite fluid; reaction very faintly alkaline; non-coagulant on being boiled anew.
						August 17th. Quite unaltered save that reaction is now neutral.
					2. Nature and treatment as above.	August 16th. Quite fluid; reaction very faintly alkaline.
						August 17th. A soft powdery coagulum and a little supernatant fluid; reaction very faintly acid.

Table III corresponds to the first table of the series, but shows the changes occurring in specimens of milk which were boiled when first procured. In order to facilitate comparison a separate column has been introduced showing the numbers cf the specimens in Table I to which those of the present table correspond. The most striking differences which the boiled, as compared with the unboiled specimens of milk, showed were in regard to reaction and coagulation. The results were not, however, of uniform character throughout, for, whilst in some cases the specimens after a period of 24 hours remained perfectly fluid and unaltered in reaction, in others, especially if they had only been once boiled, coagulation had occurred, sometimes only imperfectly, but in others fully developed and of the normal type, and the reaction, although not so highly permanently acid as is normal to unboiled specimens, was yet transitorily decidedly acid, and in some cases remained feebly so permanently.

The most curious point in regard to this difference in results was that the occurrence of coagulation and alteration in reaction appeared to be more closely related to the stratum of the sample from which the given specimens were obtained than to the fact that they had been exposed to repeated boiling or not.

This fact was first ascertained accidentally during the course of a series of operations devoted to the attempt to obtain a sufficient stock of sterilised milk to form the basis for experiments on the phenomena following inoculations of Comma-Bacilli. For some time these proved unsuccessful, due to the fact that the milk, even if boiled more than once in carefully sterilised vessels, either had coagulated spontaneously within the course of 24 hours or underwent full coagulation and separation on being boiled anew after that interval. In the course of these attempts it was observed that residual portions of milk treated with less care than the rest of the fluid, and representing the residue of the stock supply remaining in the vessel in which it had been obtained, sometimes remained unaltered when those which had been treated with special care had undergone change. Attention having thus been attracted to differences in the strata of the milk as determining the different phenomena presenting themselves under what otherwise appeared to be uniform conditions, a series of experiments was directed to the investigation of the point, and by siphoning off specimens from the upper and lower strata of identical samples of milk it was clearly shown that, whilst a single boiling sufficed to secure an abolition of the normal acid fermentation and coagulation of specimens from the lower strata, a similar treatment failed to do so with specimens taken from the top.

When the phenomenon of normal coagulation and acid fermentation occurring in spite of the specimens having been boiled first presented itself, it appeared not impossible that it was related to atmospheric conditions and especially to elevation in temperature, as it did not manifest itself in any of the first samples of the series obtained in the beginning of the year and when the temperature was relatively low. The earliest example in which the phenomenon manifested itself was in a sample of milk obtained on the 8th of March, whilst in May, at a time when the temperature was very high, it occurred as a normal event. On the recognition of the different relations which different strata of one and the same specimen of milk bear to the occurrence of the phenomena, it appeared, however, probable that the different results obtained in connection with the different periods of year were really related to these, and not to conditions of temperature. The earlier samples were portions of milk introduced directly into sterilised tubes from that presented for use at table, and may very well in some cases have been derived from the lower stratum of the entire bulk of the fluid representing the house-supply; in only one of them—that obtained on the 8th March—did coagulation and acid fermentation occur subsequent to boiling. The samples from the 15th March onwards were obtained in large quantity and direct from the bazaar, from the milkman who furnished the house, or in two instances from the Zoological Garden, and until attention had been attracted to the influence of strata, the individual specimens kept under observation were obtained by pouring off milk from the stock-supply and, therefore, must have corresponded with superficial strata of it.

This difference in the phenomena presented by portions of milk from one and the same sample according to the level in the fluid from which they have been obtained is a very striking one. It certainly cannot be ascribed to the presence of any living ferments in the upper strata and absent in the lower ones, as the results of cultivations of boiled milk showed that in all cases, and whether the fluid had been obtained from the upper or lower strata of the sample, sterilisation had been completely effected in regard to all organisms save *Bacillus subtilis*, and that this was present throughout the entire depth. On the other hand, the occurrence of acid fermentation and coagulation is unequivocally related to the presence and multiplication of schizomycete organisms in the milk, so that it appears to be probable that the explanation of the phenomenon must lie either in the actual ferment developed by the schizomycetes and affecting the milk, having a tendency to accumulate in the upper portion of the fluid, or in peculiarities in the constitution of the latter as a material to be acted on by it. This, however, is a point which will be again referred to in connection with an account of the results of cultivations of specimens of boiled milk.

Ordinary acid fermentation and coagulation thus does not occur in samples of the lower strata of any common mass of milk as supplied in Calcutta, if these have been once boiled so as to destroy the normal lacteal schizomycetes present in them, and if complete sterilisation be effected, the fluid remains permanently unaltered, save that in some cases a very slight and often fugitive acidity may gradually manifest itself. If, however, complete sterilisation be not effected, if the *Bacillus subtilis* surviving ordinary boiling remain in the fluid, whilst the normal acid fermentation and coagulation does not occur, coagulative changes of a different type manifest themselves at a later period. The coagulum in these cases is not of the firm consistence of that in the case of the ordinary acid fermentation, nor does it appear in the form of large masses but as a copious deposit of fine powdery flocculi, easily diffused throughout the fluid, which thus, after agitation, for a considerable time appears to casual observation as though it retained its original characters and had not undergone any coagulative change.

TABLE IV.—*Results of Plate Cultivations of Boiled Specimens of Milk from various sources.*

Number of specimen.	Number of corresponding specimen in Table II.	Date at which procured and boiled.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
I	III	Jan. 9th. Twice boiled; at 10 A.M. and 1 P.M.	House-supply.	...	1. Plate inoculation with 0·083 C.C Set in incubator.	January 10th.	January 11th Crowded with colonies of *Bacillus subtilis*: a pure cultivation of that species.

Number of specimen.	Number of corresponding specimen in Table II.	Date at which procured and boiled.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
		Jan. 10th. Twice boiled at 10 A.M. and 1 P.M.			2. Plate inoculated with 0·083 C.C. Set in incubator.	January 11th.	January 13th. A solitary colony of *Bacillus subtilis.*
		Jan. 11th Treated as above.		Jan. 11th. Faintly acid.	3. Plate inoculated with 0·083 C.C. Set in incubator.	January 12th.	January 13th, and subsequently quite sterile.
II	IV	Jan. 16th. Twice boiled; at 10 A.M. and 1 P.M.	House-supply.	Jan. 16th. Neutral.	1. Plate treated as above.	January 17th.	January 18th. A pure cultivation of *Bacillus subtilis.*
		Jan. 17th. Treated as above.		Feb 27th. Neutral.	2. Plate treated as above.	Feb. 27th.	February 28th, and subsequently remained absolutely sterile.
III	VI	March 8th. Once boiled.	„	March 8th. Neutral.	1. Plate treated as above.	March 9th.	March 10th. A pure cultivation of multitudes of colonies of *Bacillus subtilis:* smell faint and mouldy.
IV	VII	March 10th. Once boiled.	„	March 10th. Neutral.	1. Plate set as above.	March 11th.	March 12th. Surface in greater part covered by a continuous, slightly wrinkled stratum of *Bacillus subtilis.*
		March 11th. Once boiled.		March 11th. Neutral.	2. Plate set as above.	March 12th.	March 13th. Absolutely sterile.
V	VIII	March 12th. Once boiled.	„	March 12th. Neutral.	1. Plate set as above.	March 13th.	March 14th. Containing colonies of *Bacillus subtilis* in the proportion of 271,320 per C.C: a pure cultivation.
		March 13th. Once boiled.		March 13th. Neutral.	2. Plate set as above.	March 14th.	March 15th. Sterile.
VI	...	March 15th. Once boiled.	Bazaar.	March 15th. Neutral. March 16th. Acid.	1. Plate inoculated with 0·058 C.C. and set in incubator.	March 16th.	March 17th. Surface covered by a thick, continuous, dry, tough coating of *Bacillus subtilis.*
VII	IX	May 9th. Once boiled.	Zoological Garden.	May 9th. Neutral.	1. Plate set as above.	May 10th.	May 11th. Absolutely crowded with colonies of *Bacillus subtilis;* smell smoky.
		May 10th, 11th, 12th, 13th, 14th. Once boiled.			2, 3, 4, 5, 6, Plates set as above.	May 11th, 12th, 13th, 14th, 15th.	May 12th, 13th, 14th, 15th, 16th. Abundant crops of *Bacillus subtilis.*

Number of specimen.	Number of corresponding specimen in Table II.	Date at which procured and boiled.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
		May 15th. Twice boiled; 9 A.M. and 12-30 P.M.			7. Plate inoculated with 0·058 C. C. and set in incubator.	May 16th.	May 17th, and subsequently absolutely sterile.
VIII	...	May 9th. Once boiled.	Zoological Garden.	...	1. Plate set as above.	May 10th.	May 11th. Covered by a continuous, dense, dry, wrinkled, thick coating of *Bacillus subtilis;* smell musty.
IX	X	May 21st Once boiled.	House-supply.	May 21st. Faintly acid; neutral on drying.	Ditto .	May 22nd.	May 23rd. Full of colonies of *Bacillus subtilis.*
X	XI	May 23rd. Once boiled.	„	May 23rd. Neutral, becoming faintly alkaline.	Ditto .	May 24th.	May 25th. Crowded with colonies of *Bacillus subtilis,* but not a pure cultivation, a certain number of colonies of a large Micrococcus being also present.
XI	XIII	May 27th. Once boiled.	Bazaar	May 27th. As above. May 28th Acid, becoming neutral.	Ditto .	May 28th.	May 29th. Absolutely crowded with colonies of *Bacillus subtilis.*
XII	XIV	May 29th. Once boiled.	„	...	Ditto .	May 30th.	May 31st. Full of colonies of *Bacillus subtilis.*
XIII	XV	May 31st. Once boiled.	„	...	Ditto .	June 1st	June 2nd. An abundant growth of *Bacillus subtilis.*
		June 1st, 2nd, 3rd, 4th, 5th. Once boiled.			2, 3, 4, 5, 6. Plates set as above.	June 2nd, 3rd. 4th, 5th, 6th.	Yielded abundant crops of *Bacillus subtilis.*
XIV	...	June 7th. Boiled; 10 A.M. and 6 P.M.	„	Neutral .	1. Plate set as above.	June 8th.	June 9th. Crowded with small colonies of *Bacillus subtilis.*
		June 8th and 9th. Treated as above.			2. Plate set as above.	June 9th.	June 10th. Ditto ditto.
					3. Plate set as above.	June 11th.	June 12th. Perfectly sterile.
		June 10th, 11th. Kept for some hours in a boiling water-bath.			4. Plate set as above.	June 12th.	„ 13th. „ „
					5. Plate set as above.	June 13th.	„ 14th. „ „
XV	...	July 20th. Once boiled.	„	„ .	1. Plate set: inoculated 0·03 C. C. and set in incubator.	July 21st.	July 22nd. Covered by an almost universally confluent stratum of colonies of *Bacillus subtilis.*

Number of specimen.	Number of corresponding specimen in Table II.	Date at which procured and boiled.	Source.	Reaction.	Nature of cultivation.	Date at which set.	RESULT.
XVI	XVIII	July 25th. Once boiled.	Bazaar	Neutral .	1. Plate inoculated with one common needle dipped in a sterilised, neutral solution of salt into which one looped needle dipped in the milk had been introduced.	July 31st.	August 1st. Covered by a continuous stratum of *Bacillus subtilis.*
XVII	...	August 3rd. Once boiled.	„	August 3rd. Neutral. August 8th . Faintly acid.	1. Plate set as above.	August 8th.	August 9th. Covered by a continuous stratum of *Bacillus subtilis.*
XVIII	XX	August 15th. Once boiled.	„	Neutral .	Ditto .	August 16th.	August 17th. An abundant pure cultivation of *Bacillus subtilis.*

Table IV bears the same relation to Table II that the previous Table bears to Table I. It shows the results of a series of cultivations of boiled specimens of milk. These are very uniform and show clearly that a single and brief boiling is sufficient to clear normal samples of the fluid from all living organisms save *Bacillus subtilis*, which then multiplies with excessive rapidity. The numbers of colonies of the latter organism occurring in cultivations of boiled milk are illustrated in the four photographs on the right side of the plate. In connection with *Bacillus subtilis* there are two phenomena in these experiments specially noteworthy—first, the evidence which is afforded that the other schizomycete organisms normally occurring in milk in Calcutta exert a repressive action on the development of *Bacillus subtilis*, and, second, the variations occurring in regard to the persistence of *Bacillus subtilis* in different specimens of milk. The repressive influence of the other normal schizomycetes inhabiting the fluid on the development of *Bacillus subtilis* was very clearly demonstrated throughout the entire series, the amount of the latter species developed in any cultivation of unboiled milk being extremely limited, and its appearance almost entirely confined to cultivations set immediately after the samples of milk were obtained, and, therefore, at a stage when they contained relatively small numbers of the other common schizomycetes as compared with those present at later periods. The results obtained in the case of the eighth cultivation recorded in the present Table are specially instructive in this respect as compared with those recorded in regard to corresponding cultivations of a specimen of the same sample of milk which had not been boiled. The latter are entered under No. IX of Table II. It will be seen that while the two cultivations of the unboiled specimen failed to show any evidence of the presence of *Bacillus subtilis*, that of the boiled specimen within 24 hours became covered by an enormous growth of the species in the form of a continuous, thick, dry, wrinkled stratum covering the entire surface of the plate. Judging by the very feeble development manifesting itself in any of the cultivations of unboiled samples of milk, it would not appear probable that the proportion of rods and spores of *Bacillus subtilis* originally present in the fluid is considerable, as, were it so, the species would probably show more evidence of success in the struggle for existence with its companions. Direct evidence of this was obtained by means of a series of successive cultivations of one sample of boiled milk, the results of which are recorded in Table V—

TABLE V.—*Results of Successive Cultivations of a Specimen of Boiled Milk.*

Nature of specimen.	Source, and Date when obtained and boiled.	No. of plate cultivation.	Time at which set.	RESULTS.
Milk once boiled in a sterilised tube; reaction neutral.	Bazaar-September 6th.	...	......	September 7th. Quite fluid; reaction neutral. September 8th. Quite fluid; reaction faintly acid. September 9th. Separated into fluid and large, loose coagulum of powdery flocculi; reaction neutral, very faintly alkaline on drying. September 10th. Three distinct strata present; 1st at the surface, a thick layer of cream; 2nd, intermediate, a large amount of yellowish fluid; 3rd inferiorly, an abundant deposit of powdery, white, flocculi; easily diffused by shaking, when the milk for a considerable time resumed a normal appearance; reaction very faintly acid.
		1	Immediately after boiling; 0·03 C. C. inoculated.	September 7th. Quite sterile.
		2	September 7th, 24 hours after boiling; 0·03 C. C. inoculated.	September 8th. A pure and very abundant growth of *Bacillus subtilis* in the form of innumerable, discrete colonies, and a generally diffused, tough, dry film extending over the whole surface.
		3	September 9th, 72 hours after boiling; inoculated by a needle dipped in sterilised salt solution inoculated by one looped needle from the milk.	September 10th. A pure and crowded growth of *Bacillus subtilis.*

In all other cultivations of boiled milk periods of from 18 to 24 hours elapsed between the boiling of the milk and the establishment of the cultivation, but in this series the first cultivation was set within 10 minutes from the boiling, and, although the amount inoculated and the subsequent treatment were identical with those which in other cases were invariably followed by an enormous crop of *Bacillus subtilis*, the cultivation in this case proved absolutely sterile. This, however, was not the case with a similar cultivation set 24 hours later, for here the usual result followed and the plate became occupied by innumerable discrete colonies and an abundant, diffuse, superficial growth of *Bacillus subtilis*. It would appear, thus, that only a relatively small number of the spores of *Bacillus subtilis* are normally present in ordinary samples of milk, and that these are incapable of successfully holding their own in the struggle for existence with the other schizomycete organisms with which they are normally associated, but that, if relieved from the struggle by the destruction of their rivals by means of boiling, they find the medium well adapted to their development and multiplication. The sample of boiled milk from which the above results were obtained also afforded an excellent demonstration of the series of changes which occur in specimens of boiled but not sterilised milk—in other words, in specimens in which the organisms whose multiplication is ordinarily associated with the acid fermentation and common coagulation of the fluid have been destroyed, but in which rapid multiplication of *Bacillus subtilis* goes on. After a lapse of 24 hours from the period of boiling, the milk remained perfectly fluid, but showed a slight change in reaction, its previous neutrality having been replaced by feeble acidity. Forty-eight hours after the commencement of the experiment the reaction was neutral at first, becoming faintly alkaline on drying, and coagulation and partial separation had already occurred. The coagulum was of the peculiar, finely pulverulent character normal to specimens of boiled milk. On the following day separation of coagulum and fluid had advanced considerably and the reaction was now very faintly acid. The alternation of feebly developed acidity and alkalinity which occurred in this case is a phenomenon which frequently manifests itself in connection with samples of milk which are undergoing change in connection with the growth and multiplication of *Bacillus subtilis* within them.

The variations which presented themselves in regard to the persistence of *Bacillus subtilis*, in specimens of milk which were repeatedly boiled, at first sight appear very remarkable; two successive boilings in some cases securing sterilisation, as in specimens Nos. IV and V of the present table, whilst in others, such as that of No. XIII, six successive boilings completely failed to get rid of or diminish the numbers of *Bacillus subtilis* appearing in subsequent cultivations. This holds good of ordinary brief exposure to the influence of boiling over an open flame only, for a single prolonged exposure of several hours' duration in a boiling water-bath, although in all probability the actual temperature in such circumstances is not so high as in the previous set of cases, appears invariably to be followed by complete sterilisation. These phenomena are, no doubt, due merely to the presence and properties of the spores of the Bacillus. The exposure to the continued elevation of the water-bath suffices at once to destroy the vitality, not merely of the developed rods, as brief boiing does, but also that of all the spores which may simultaneously be present. The precise effect of mere brief boiling will vary necessarily according to the absence or presence of spores. Should the medium be exposed to brief boiling at a time when all pre-existent spores have germinated, and before a new crop has matured, a single exposure will be sufficient to secure sterilisation; but, if periods of boiling do not correspond with periods of absence of spores, the treatment might, of course, be repeated indefinitely without securing the occurrence of sterilisation. The tendency to produce spores and the rate with which the process is carried out in individual cases probably varies, not merely in relation to variations in external conditions to which the Bacilli are exposed, but also in relation to hereditary tendencies in the latter themselves, so that even in cases where external conditions are absolutely alike, a uniform result in respect of amount and rate of spore-formation need not necessarily present itself. So far as it was possible, the specimens of milk forming the greater bulk of this series were exposed to exactly similar conditions, the similarity of thermal ones, at all events, being absolutely secured by the use of an incubator retained at one steady temperature throughout, and yet treatment, which at one time of year seemed to be quite effectual in securing sterilisation, at another entirely failed to do so. That indefinitely repeated exposures of the medium to boiling at relatively brief intervals should in some cases fail to secure sterilisation, whilst two boilings at wider intervals secure it in others, must surely be related to presence or absence of spores. It might at first sight seem that frequent repetition of boiling must inevitably tell in favour of sterilisation, but, of course, it need not do so unless the rhythm of the cycle of spore-formation and evolution of Bacilli from the spores falls in such fashion that periods of boiling and of absence of spores coincide with one another. Unless this be so, the rapid repetition of the boiling in place of telling more favourably for sterilisation than less frequent treatment may act in precisely opposite fashion. A single boiling falling exactly at a time of absence of spores will serve to secure sterilisation, many repeated boilings, if they do not coincide with periods of such character, will, of course, fail to do so, and the very frequency of repetition may secure such want of coincidence.

When, as the result of experiments of the nature of those recorded in the previous tables, some definite idea of the normal phenomena presenting themselves in specimens of the ordinary milk-supply in Calcutta had been obtained, a special investigation of the behaviour of Comma-Bacilli in the latter were carried out. Considerable delay occurred ere these could be initiated, and this, taking the locality and time of year—Calcutta and the months of May and June—into account, was noteworthy, seeing that it was caused by the want of a supply of the Bacilli. The laboratory was closed from the middle of March until the 6th of May, and during the interval all the tube-cultivations of Comma-Bacilli, even those specially set on the 18th of March, had completely died out. This was not at first sight regarded as a matter of any importance, as it appeared desirable to carry out the experiments with cultivations of the Bacilli not too far removed in succession from their original choleraic source, and, taking the time of year into account, there appeared to be no likelihood of any difficulty being encountered in securing a fresh stock. Steps were at once taken to obtain supplies of choleraic materials. These were at first derived from the Medical College Hospital, but, although derived from cases regarded and recorded as cases of cholera, and more than one of which terminated fatally, in no instance could any Comma-Bacilli be obtained from them in spite of most careful and repeated cultivations. The materials derived from this source proving so unfruitful, materials were next obtained from the Sealdah Hospital. These, as in the previous case, were derived from cases which were regarded as typical cases of cholera, but for some time the results were identical with those recorded of the other materials—they failed absolutely to show any evidences of containing Comma-Bacilli. This persistent and uniform absence of Comma-Bacilli in cultivations of materials of affirmed and apparent choleraic nature was so remarkable and unexpected that a suspicion not unnaturally arose in regard to the quality of the materials forming the basis of the cultivations, which, as usual during the hot season, consisted of Agar-Agar beef juice gelatine. Several successive samples of this were accordingly prepared with special care, but all alike continued to furnish the same negative results. The quality of the *Peptonum siccum* employed in the preparation of the medium was next specially suspected, as that in use, although most carefully preserved, had been in the laboratory for some years, and a fresh supply was accordingly ordered out from Berlin. At length, however, before this had arrived, one out of 11 successive specimens of choleraic materials, derived from as many reputed cases of the disease, yielded a crop of Comma-Bacilli on cultivation, It was obtained from the Sealdah Hospital, and a plate-cultivation inoculated from it in the usual fashion yielded a practically pure crop consisting of innumerable colonies of characteristic Comma-Bacilli. This removed suspicion from one of the successive supplies of the cultivating medium which had been prepared, and on trying the others as bases for the growth of the Bacilli which had now been obtained, it was definitely ascertained that the previous consistent failure in the appearance of the Commas was not due to any error in the constitution of the various media, as they all alike now yielded abundant crops of characteristic Comma-Bacilli.

The interpretation which will be put on this phenomenon will, of course, vary with the views, which are entertained by those considering it, in regard to the relation existent between Comma-Bacilli and cholera. Those who are convinced that cholera is caused by the presence of Comma-Bacilli within the intestinal canal will naturally regard it as the result of imperfect diagnosis; those, on the other hand, who regard the presence of numerous Comma-Bacilli in the intestinal contents of choleraic patients as a result, not a cause, of the morbid state, will see in it a confirmation of their opinion. In any case, it is a phenomenon calculated to excite a certain reserve in the acceptation of a belief in the absolute value of the presence of cultivable Comma-Bacilli as specially diagnostic of the nature of any case as a true case of cholera. If we place implicit faith in the diagnostic value of the presence of cultivable Comma-Bacilli in the alvine discharges, we must believe that the diagnosis of cholera in Calcutta is very imperfect, and that the registration of the prevalence of the disease is liable to be correspondingly greatly in excess of its actual prevalence, but if we do not, we can only see additional ground for scepticism in regard to the essential causal relation existent between the presence of the Bacillus within the intestinal tract and the development of choleraic phenomena in the organism.

Returning from this digression, it remains to consider the phenomena manifesting themselves in cases where Comma-Bacilli have gained access to samples of milk. Three distinct series of experiments specially relating to these were carried out. In the first the results following the access of Comma-Bacilli to samples of milk of ordinary quality, as represented by the milk commonly procurable in the Calcutta bazars, were the special subject of investigation; in the second the enquiry was directed to the phenomena presenting themselves in cases where the common normal lacteal schizomycetes had been partially removed by means of boiling, so that the intrusive Comma-Bacilli had no longer to contend with any living elements in the milk save *Bacillus subtilis*; in the third, the effects manifesting themselves in cases where the milk, in place of having its schizomycete population merely reduced, as in the previous series of experiments, has had it absolutely abolished ere inoculation of Comma-Bacilli—in other words the behaviour of sterilised milk as a medium for Comma-Bacilli—was particularly enquired into. The result of these experiments are summarised in the three following tables.

The first of these, Table VI, deals with the phenomena attendant on the introduction of large masses of Comma-Bacilli into common samples of milk, which had not been exposed to any special preliminary treatment.

TABLE VI.—Results of inoculation of specimens of unboiled milk with Comma-Bacilli.

No. of specimen.	Nos. of corresponding specimens in Tables I & II.		Nature and treatment of specimen.	Date of inoculation.	Nature of cultivations.	Date at which set.	RESULTS.
	I.	II.					
I	XIV	XVII	Milk in a sterilised tube; inoculated with 0·059 of sterilised salt solution full of Comma-Bacilli from a tube of 48 hours' growth	15th July.	......	...	16th July. Coagulated, but not fully separated into coagulum and fluid; reaction permanently acid; smell caseous. 17th July. Fully coagulated and separated; reaction strongly and permanently acid. 19th July. Reaction violently acid smell very faint.
					1. Plate; inoculated by a looped needle.	16th July.	17th July. Smell mouldy, non-choleraic; crowded with colonies of the common, lacteal, oval and straight Bacilli; no colonies of commas to be found.
					2. Plate; inoculated by a common needle.	17th July.	18th July. Smell mouldy; almost a pure cultivation of the common, small, straight, lacteal Bacilli; *Bacillus subtilis* present in very small amount and apparently in the form of a thin diffused surface growth.
					3. Plate; inoculated by a common needle.	19th July.	21st July. Only two colonies, and these both of large, lacteal Micrococcus.
II	...	...	Milk in a sterilised tube; inoculated as above with 0·03 solution containing Comma-Bacilli of 48 hours' growth.	20th July.	......	...	21st July. Coagulated and partly separated; reaction acid. 23rd July. Reaction strongly acid.
					1. Plate; inoculated by a needle dipped in sterilised salt solution, inoculated by a looped needle from the milk.	21st July.	22nd July. An abundance of colonies present; 3 distinct species; 1st bluish and flattened, consisting of the common, small, straight, lacteal Bacilli; 2nd, prominent and yellowish, consisting of common, lacteal, oval Bacilli; 3rd, two colonies in the form of greenish yellow superficial patches, consisting of minute, straight Bacilli.
					2. Plate; inoculated as above.	23rd July.	24th July. Numerous colonies of common, lacteal, oval Bacilli and Micrococcus.
III	XV	XVIII	Milk treated as above.	25th July.	......	...	26th July. Exactly in the same condition as a corresponding non-inoculated specimen of the same milk; in very soft coagulum but not yet separated; reaction permanently acid.

No. of specimen.	Nos. of corresponding specimens in Tables I & II.		Nature and treatment of specimens.	Date of inoculation.	Nature of cultivations.	Date at which set.	RESULTS.
	I.	II.					
							27th July. Fully coagulated and separated; reaction strongly acid.
							28th July. Reaction violently acid.
					1. Plate; inoculated by a needle dipped in sterilised salt solution inoculated by a looped needle from the milk.	26th July.	27th July. Smell musty, non-choleraic; crowded with colonies of the common lacteal Schizomycetes; not a trace of Comma-Bacilli
					2. Plate; inoculated as above.	27th July.	28th July. Numerous colonies of common lacteal schizomycetes; not a trace of Comma-Bacilli.
IV	XVI	XIX	Milk in a sterilised tube; inoculated with 0·03 solution, containing Comma-Bacilli of 48 hours' growth.	29th July.	......	...	30th July. Exactly like its companion, non-inoculated tube; reaction acid; slightly frothed; at first in soft loose coagulum, afterwards undergoing separation.
					1. Plate; inoculated as above.	30th July.	31st July. Exactly like its companion plate of non-inoculated milk; smell mouldy; numerous colonies of common lacteal schizomycetes; no Comma-Bacilli.
V	XVII	...	,,	3rd Aug.	......	...	5th July. Strongly acid; fully coagulated and separated.
					1. Plate; inoculated as above.	4th July.	5th July. Smell mouldy; numerous colonies of common lacteal schizomycetes; none of Comma-Bacilli.
					2. Plate; inoculated as above.	5th July.	6th July. An abundant crop of colonies of common lacteal schizomycetes; no Comma-Bacilli.
VI	XVIII	XX	Milk treated as above, save that the Comma-Bacilli were derived from a cultivation of only 24 hours' standing. The amount of the solution containing Comma-Bacilli was equal to about 0·0029 of the bulk of milk.	15th Aug.	......	...	16th August. Exactly in the same condition as its companion specimen of non-inoculated milk; strongly acid; coagulated, and partly separated; somewhat frothed at the top.
					1. Plate; inoculated as above.	16th Aug.	17th August. Exactly like a plate-cultivation of the same milk set on the 15th previous to inoculation, save that the number of colonies of common lacteal schizomycetes is considerably greater; not a trace of colonies of Comma-Bacilli.

The six experiments recorded in this Table were practically identical in nature and results. The actual quantity of fluid containing Comma-Bacilli introduced into the milk varied slightly in the different experiments, and so did the precise amount of the milk. In one case where the point was accurately determined, the amount of the fluid added to the milk was equivalent to about one three-hundredth of its bulk. The fluid which was employed was in all cases crowded with enormous numbers of healthy Comma-Bacilli, the amount of them being indicated indirectly by the opalescent character of the fluid, and being directly ascertained by means of cover-glass preparations, whilst their quality was determined by observations of the results following their cultivation in various sterilised media, such as Agar-Agar beef-juice gelatine and sterilised samples of milk. The fluid was in all cases prepared alike, a small quantity of freshly sterilised solution of common salt being introduced into a tube-cultivation of Comma-Bacilli of 24 or 48 hours' standing, and containing a great growth of the organism diffused over the oblique surface of the medium. Thorough shaking then sufficed to wash off the greater part of the growth and to diffuse the Bacilli through the salt solution, which coincidently exchanged its previous transparency for opalescence. A little of the opalescent fluid was then removed by means of a freshly sterilised and calibrated pipette, and a single drop from the latter added to the sample of milk under investigation.

This was contained in a sterilised and plugged tube, which was then set in the incubator at 37° C., and subsequently examined from time to time. The examination dealt in the first place with the general phenomena of change manifesting themselves in the milk, and, secondly, with the evidence as to the persistent presence and multiplication of the normal lacteal schizomycetes present in it originally and of the inoculated Comma-Bacilli. The phenomena presenting themselves in the inoculated specimens were compared with those occurring in corresponding specimens of milk derived from the same primary sample, which had not been inoculated, but which had otherwise been exposed to like conditions.

In regard to the general changes manifesting themselves in the milk, the inoculated specimens showed no appreciable differences from their non-inoculated compeers, and the presence of the Comma-Bacilli did not appear to have interfered with the progress of the acid fermentation and coagulation normal to ordinary samples of milk. Within the course of 24 hours coagulation had occurred in every case, and the reaction had assumed various degrees of permanent acidity. Separation of the coagula and fluid had generally begun to occur, and in every case was fully carried out within 48 hours from the commencement of the experiment, the acidity at the same time becoming very strongly pronounced.

The cultivations of the inoculated milk also showed nothing to distinguish them from those derived from the fluid in its normal condition. An abundant development of colonies of the common lacteal schizomycetes invariably occurred within 24 hours in plate-cultivations of the milk which had been set 24 hours subsequent to the inoculation of the latter, but in no case could any development of colonies of Comma-Bacilli be detected, and the general result of the series of experiments was thus to show that the Bacilli, even when introduced in very large numbers, do not find conditions in ordinary samples of milk favourable to their multiplication or even to their continued survival for any considerable time.

So much having been ascertained, it next appeared to be desirable to follow the course of events subsequent to inoculation somewhat more closely, and a series of cultivations derived from one and the same sample of milk, but set at successive periods shortly after inoculation had been effected, was accordingly carried out. The results of these are embodied in Table VII :—

TABLE *VII.—Results of successive Cultivations of a Specimen of Unboiled Milk inoculated with Comma-Bacilli.*

No. of specimen in Table VI.	Date and time at which inoculated.	No. of Plate-cultivation.	Date and time at which set.	RESULTS.
V.—Milk in a sterilised tube inoculated with 0·03 C.C. sterilised salt solution full of the Comma-Bacilli of a tube-cultivation of 48 hours' standing.	August 3rd, 8 A.M.	1	August 3rd, 10 A.M. Plate; inoculated by a looped needle dipped in the milk.	August 4th. Numerous colonies of lacteal schizomycetes, and a few of characteristic Comma-Bacilli; smell non-choleraic.
		2	August 3rd, 12 noon. Plate set as abo	August 4th. A greatly increased number of colonies of common lacteal schizomycetes, and a somewhat larger number of colonies of Comma-Bacilli; smell non-choleraic.
Reaction at first neutral; at noon faintly acid.	...	3	August 3rd, 2 P.M. Plate set as above.	August 4th. Excessive increase in the number of colonies of common lacteal schizomycetes, and great diminution in the number of colonies of Comma-Bacilli; smell non-choleraic.
August 5th. Strongly acid; fully coagulated and separated.	...	4	August 3rd, 6 P.M. Plate; inoculated as above.	August 4th. Absolutely crowded with colonies of common lacteal schizomycetes; only a single colony of Comma-Bacilli detected; smell non-choleraic.
		5	August 4th, 10 A.M. Plate; inoculated by a common needle.	August 5th. Numerous colonies of common lacteal schizomycetes; not a trace of Comma-Bacilli; smell non-choleraic.

Inoculation was performed at 8 A.M., and successive cultivations set at intervals of 2, 4, 6, 10 and 24 hours thereafter. The first four of these showed evidences of excessive multiplication of common lacteal schizomycetes occurring within the first 10 hours of the experiment, and they also showed that Comma-Bacilli survived in the fluid for a like period. So far as the evidence goes, it would appear to indicate, not mere survival, but also multiplication of the Comma-Bacilli as occurring for some time subsequent to their introduction into the milk, for a larger number of colonies of the organism were present in the cultivation set at four hours after inoculation than in that set only two hours after it. The period of multiplication would seem, however, to be a brief one, for the third cultivation of the series, set at six hours subsequent to inoculation, showed a diminution in the number of colonies, while that set 10 hours after inoculation contained only a single recognisable one. The last cultivation of the series gave the normal result for cultivations established after the lapse of 24 hours from the period of inoculation, in containing large numbers of colonies of common lacteal schizomycetes and a complete absence of Comma-Bacilli. The cessation of multiplication and onset of disappearance of the Bacilli would appear to have been coincident with the development of acidity in the medium—permanent, although primarily only faint, acidity having replaced neutrality within four hours subsequent to the time of inoculation, and acid fermentation having thereafter progressed rapidly so as to cause the reaction 24 hours after inoculation to be strongly acid, and the milk to be fully coagulated and separated.

Milk in its ordinary condition, therefore, appears to be anything but a favourable medium for Comma-Bacilli, the rapidly developed acid fermentation normally setting in in it connected with the presence and multiplication of other schizomycetes tending to arrest their multiplication and presently to destroy their vitality. That it is the presence of the common lacteal schizomycetes and the fermentive changes developed in the fluid in connection with it which is the actually determinant of the repression of multiplication and vitality of the Comma-Bacilli is clearly shown by the data recorded in the two next tables, which deal with the results following the introduction of Comma-Bacilli into boiled and sterilised samples of milk—

TABLE *VIII.—Results of Inoculations of Specimens of Boiled Milk with Comma-Bacilli.*

No. of specimen.	Nos. of corresponding specimens in Tables III & IV.		Nature and treatment of specimen.	Date of inoculation.	Nature of cultivations.	Date at which set.	RESULTS.
	III.	IV.					
I	XVI	XV	Milk boiled in a sterilised tube, July 20th.	July 22nd.	Inoculated with 0·03 of sterilised salt solution full of Comma-Bacilli of 48 hours' growth.	...	July 23rd. Reaction faintly acid, completely coagulated into a large, white, powdery deposit and clear colourless fluid. July 24th. Reaction almost neutral.

No. of specimen.	Nos. of corresponding specimens in Tables III & IV.		Nature and treatment of specimen.	Date of inoculation.	Nature of cultivations.	Date at which set.	RESULTS.
	III.	IV.					
					1. Plate; inoculated with a common needle dipped in sterilised salt solution inoculated by one looped needle dipped in the milk.	July, 23rd.	July 24th. Full of large colonies of Comma-Bacilli; scattered and unhealthy looking rods of *Bacillus subtilis* also present, seemingly due to the presence of a thin, diffuse, surface growth; smell strongly choleraic; practically a cultivation of Comma-Bacilli.
II	XVIII	...	Milk boiled in a sterilised tube, July 29th.	July 31st.	Inoculated as above.	...	August 1st. Reaction neutral; smell putrid; coagulated. August 6th. Reaction faintly acid at first, faintly alkaline on drying.
					1. Plate; inoculated as above.	Aug. 1st.	August 2nd. Covered with discrete and confluent colonies of Comma-Bacilli and a thin, diffuse growth of *Bacillus subtilis.*
					2. Plate; inoculated as above.	Aug. 6th.	August 7th. Apparently a pure cultivation of *Bacillus subtilis.*
III	XIX	XVII	Milk boiled in a sterilised tube, August 3rd.	Aug. 9th.	...	...	At the time of inoculation the milk was coagulated and partly separated; coagulum of the loose, pulverulent character nominally associated with *Bacillus subtilis;* full of *Bacillus subtilis;* smell strong, putrid; reaction faintly acid, almost neutral on drying.
					1. Plate; inoculated as above.	Aug. 14th.	August 15th. Covered by a continuous, thick stratum of *Bacillus subtilis* and containing a limited number of colonies of large Comma-Bacilli; smell non-choleraic.
IV	XX	XVIII	Milk boiled in a sterilised tube, August 15th.	Aug. 17th.	...	...	At the time of inoculation the milk showed coagulation characteristic of the continued presence of *Bacillus subtilis,* being full of fine, loose, powdery flakes, with a little free, supernatant fluid; reaction very faintly acid. August 18th. Separation of coagulum and fluid considerably advanced; reaction neutral. August 21st. Reaction neutral. August 22nd. Reaction very faintly acid. September 29th. Reaction neutral.
					1. Plate; inoculated as above.	Aug. 18th.	August 19th. An abundant growth of colonies of Comma-Bacilli, and a partial and very thin, diffuse growth of *Bacillus subtilis.*

No. of specimen.	Nos. of corresponding specimens in Tables III & IV.		Nature and treatment of specimen.	Date of inoculation.	Nature of cultivations.	Date at which set.	Results.
	III.	IV.					
	XX	XVIII	...	...	2. Plate; inoculated as previously.	Aug, 19th.	August 20th. A very limited number of colonies of Comma-Bacilli, and one or two very thin, localised films of *Bacillus subtilis.*
					3. „ „	Aug. 20th.	August 21st. A single large colony of *Bacillus subtilis;* no Comma-Bacilli present.
					4. „ „	Aug. 21st.	August 22nd. Surface almost covered by a thick stratum of sporing *Bacillus subtilis;* one or two large colonies of small, feebly-staining Comma-Bacilli.
					5. „ „	Aug. 22nd	August 23rd. A solitary colony of large Comma-Bacilli; no *Bacillus subtilis.*
					6. „ „	Aug. 23rd.	August 24th. One or two colonies of short, thick Comma-Bacilli, and a localised very thin diffuse film of *Bacillus subtilis.*
					7. „ „	Aug. 24th.	August 26th. An abundant growth of sporing *Bacillus subtilis* and small, feebly-staining Comma-Bacilli.
					8. „ „	Aug. 31st.	September 1st. Smell very faint, non-choleraic; crowded with a diffuse growth and ill-defined colonies of small, feebly-staining, vacuolate Comma-Bacilli, and a thin diffuse growth of *Bacillus subtilis.*
					9. Plate; inoculated by one common needle dipped in the milk.	Sept. 19th.	September 20th. An abundant, mixed growth of *Bacillus subtilis* and Comma-Bacilli.

In the case of the boiled samples of milk referred to in the above table, it will be seen that inoculation with Comma-Bacilli was carried out at some time subsequent to the boiling—the interval elapsing between the two operations having amounted to 48 hours in three of the experiments and to 144 hours in the remaining one. The interval was allowed to occur in order to permit of the growth and multiplication of *Bacillus subtilis,* which normally takes place in boiled samples of milk, going on, without any possible interference on the part of the intrusive Bacilli, for such a time as would certainly secure its presence in very large amount within the medium at the period of inoculation. By following this procedure the capacity for co-existence of Comma-Bacilli with *Bacillus subtilis* in a medium already thickly populated by the latter organism was most satisfactorily determined. Apart from the length of interval between the operations of boiling and inoculation, the conduct of all the experiments was practically identical. The samples of milk, having been briefly boiled in sterilised test tubes over an open flame, were kept in the incubator until the period when inoculation was performed. During the interval they underwent the changes characteristic of specimens of milk occupied by pure growths of *Bacillus subtilis* in greater or less degree—pulverulent coagulation, frequently a tendency to slight development of acid, and in some cases the evolution of a foul putrescent odour. Inoculation was conducted in the same fashion as it was in the case of the experiments on unboiled samples of milk.

The results of cultivations subsequent to inoculation varied in character considerably, apparently in relation to the period which had elapsed between the introduction of the Comma-Bacilli into the milk and the establishment of the cultivation. Those cultivations which were set at a period of 24 hours subsequent to inoculation always yielded excessively abundant crops of colonies of characteristic Comma-Bacilli. At the same time there were evidences of apparent partial suppression in the development of *Bacillus subtilis* in the milk, as, though the cultivations were in no instance pure cultivations of Comma-Bacilli, the amount of the other species which was present was very small, the growth being confined to more or less extensive, but very thin, diffused strata spread over the surface of the gelatine. The development of *Bacillus subtilis* in cultivations set at this period resembled that manifesting itself in connection with some cultivations of unboiled and non-inoculated milk, and not that normally present in specimens of milk containing *Bacillus subtilis* alone as their living schizomycete content. The cultivations had a strong characteristic choleraic odour, and the films of *Bacillus subtilis* were so thin as in many cases to render their direct recognition almost impossible, even where their presence had been ascertained due to the contamination of preparations of the colonies of commas by distinct rods of the other species.

The presence of very large numbers of *Bacillus subtilis* in specimens of boiled milk does not, therefore, serve to prevent the occurrence of very rapid growth and multiplication of Comma-Bacilli which may gain access to the medium. There is no rapid suppression of the multiplication and vitality of the Comma-Bacilli such as is normally present in cases where the medium has not been boiled, and the intrusion of the Comma-Bacilli appears, on the contrary, to give rise to partial and temporary suppression in the development of the pre-existent schizomycete population represented by *Bacillus subtilis.*

This suppression and the excessive multiplication of Comma-Bacilli seems, however, to be of comparatively brief duration, for cultivations set after longer intervals from the period of inoculation frequently show a great reduction in number, and in some cases apparent total absence of Comma-Bacilli with a proportionate increase in the quantity of *Bacillus subtilis.* For example, in the case of Sample II of the Table, the first cultivation, which was set twenty-four hours after inoculation, yielded an excessive crop of Comma-Bacilli, and only a feeble growth of *Bacillus subtilis,* whilst the second cultivation, which was set five days later, showed a very abundant and seemingly pure growth of the latter species only. Again, in the case of Sample III, where only a single cultivation was carried out, and that five days subsequent to inoculation, the growth of *Bacillus subtilis* was excessive, and Comma-Bacilli, although not entirely absent, were present in relatively very small numbers only. This result does not, however, appear to be constant in regard to the time at which it occurs, for in Sample IV a cultivation set a fortnight after the date of inoculation showed excessive growth of Comma-Bacilli, and only a limited development of *Bacillus subtilis.* In the case of this sample an extended series of cultivations was made. The results of these so far agreed with those in the previous cases that they indicated an initial period of excessive growth of Comma-Bacilli, succeeded by a period during which a great diminution in their numbers occurred, with simultaneously increased activity in the growth of *Bacillus subtilis,* but, after this, neither persistent depression of growth of the former organisation, nor persistent excess of the latter manifested themselves. On the contrary, the successive cultivations, which were carried out subsequently, appeared to indicate an alternation of periods of relatively excessive and depressed activity in the case of both species alike. The results in this instance clearly showed that there is not necessarily any rapid suppression of Comma-Bacilli in association with *Bacillus subtilis* in samples of boiled milk, for the fluid continued to yield abundance of both species, up at all events to the 19th September, when the last cultivation of the series was carried out, or up to a period of almost five weeks subsequent to inoculation.

The general results of this series of experiments are to show that boiled milk affords a much more favourable medium for the growth of Comma-Bacilli than the unboiled fluid does, providing conditions certainly favouring excessive multiplication of the species for some time and not definitely precluding its continued presence in large numbers and in a living state for a very considerable period.

The last set of experiments were devoted to the investigation of the phenomena following introduction of Comma-Bacilli into specimens of milk which had not merely been boiled but actually sterilised, and the results are embodied in Table IX.

PLATE IX.—*Results of Inoculations of Specimens of Sterilised Milk with Comma-Bacilli.*

No. of specimen.	Nos. of corresponding specimens in Tables I & III.		Nature and treatment of specimens.	Date of inoculation.	Nature of cultivations.	Date of inoculation.	REMARKS.
	I.	III.					
I	XIII	XV	Sample of milk in a tube, sterilised between 7th and 10th June. Reaction faintly acid, becoming almost neutral on drying; odourless.	July 11th.	Inoculated with 0·059 of sterilised salt solution containing Comma-Bacilli of 24 hours' growth.	...	July 12th. Quite fluid; reaction faintly alkaline; containing numerous Comma-Bacilli, many of them of large size and vacuolate.
					1. Plate; inoculated by one looped needle dipped in the milk	July 12th.	July 13th. Smell strongly choleraic, crowded with colonies of Comma-Bacilli, the majority of which were short and thick.
					2. Plate; inoculated as above.	July 15th	July 16th. Absolutely crowded with colonies of Comma-Bacilli.
					3. Plate; inoculated as above.	July 24th.	July 25th. Absolutely sterile.
II	„	„	As above .	July 15th.	Inoculated as above, save that the Comma-Bacilli were derived from a cultivation of 48 hours' standing.	...	July 25th. Coagulated; coagula soft, powdery.
					1. Plate; inoculated by one common needle, dipped in the milk.	July 17th.	July 18th. Smell choleraic; crowded with colonies of Comma-Bacilli; the latter generally very short.
					2. Plate; inoculated with 0·03 of the milk.	July 25th.	July 26th Crowded with colonies of Comma-Bacilli.
					3. Plate; inoculated by one looped needle, dipped in the milk.	July 29th.	July 30th. Crowded with colonies of Comma-Bacilli.
III	„	„	As above .	Aug. 9th.	Inoculated as above; the Comma-Bacilli from a tube of 48 hours' growth.	...	August 14th reduced to a soft gelatinous clot, but with hardly any separation of fluid; smell very faint, mouldy.

No. of specimen.	Nos. of corresponding specimens in Tables I & III.		Nature and treatment of specimens.	Date of inoculation.	Nature of cultivations.	Date at which set.	REMARKS.
	I	III					
	XIII	XV	...	...	1. Plate; inoculated by a looped needle dipped in sterilised salt solution which had been previously inoculated by a looped needle, dipped in the milk.	Aug. 15th.	August 16th. An enormous and pure growth of Comma-Bacilli.
					2. Plate; treated as above.	Aug. 24th	August 26th. No Comma-Bacilli; an abundant growth of intrusive *Bacillus subtilis.*
					3. Plate; treated as above.	Aug. 30th	August 31st. Exactly like the previous plate.

The milk in all the experiments consisted of sterilised specimens of the sample numbered XV in Table III, and which were experimentally shown to be completely sterilised on the 10th of June, cultivations from that time onwards failing to show the presence of any living elements in it. Up to the present time, September 19th, the specimens of it which yet remain show absolutely no change save a diminution in bulk due to gradual evaporative loss. They retain their fluidity completely, save for the presence of a thin dry tough skin which has gradually formed on the surface as evaporation has progressed. The reaction is in some instances faintly acid at first, becoming neutral on drying, and in others absolutely neutral throughout, and were it not for the presence of the superficial film above described, and the slightly brownish tinge which they acquired as the result of the prolonged exposure to an elevated temperature during the process of sterilisation, they present all the essential characters of specimens of fresh milk.

The inoculation of those specimens which formed the subjects of special experiment was carried out in the same fashion as in the cases where fresh or merely boiled specimens of milk were dealt with. The introduction of Comma-Bacilli was ultimately followed by coagulative changes in the fluid. These were unconnected with any conspicuous alterations in its re-action, and the resultant coagula rather resembled in character those associated with the fermentive changes accompanying the persistent presence of *Bacillus subtilis* than those occurring in the case of the ordinary spontaneous acid fermentation and coagulation of common specimens of milk. In one case, where the fluid was directly examined in regard to its content of Comma-Bacilli at a period 24 hours subsequent to inoculation, the cover-glass preparations showed the presence of numerous specimens of them. Cultivations also afforded unequivocal evidence of the occurrence of their excessive multiplication in the fluid subsequent to their introduction. This, moreover, was not limited to periods shortly following inoculation, as in the case of merely boiled specimens, but appeared to persist continuously for a very considerable time. For example, in the case of sample II, cultivations set at 48 hours, ten days, and a fortnight subsequent to inoculation all alike produced excessive growths of Comma-Bacilli. The effect of freedom from a struggle for existence is very clearly indicated by this phenomenon, and even more emphatically when we compare the results in this instance with those recorded in the case of sample III. In the first instance the medium throughout remained free of any schizomycetes save the inoculated Comma-Bacilli, in the second an intrusive organism in the shape of *Bacillus subtilis* accidentally entered the medium at some period subsequent to inoculation. In the first instance Comma-Bacilli remained in the medium in excessive numbers up to a fortnight subsequent to inoculation; in the second they were present in great numbers six days subsequent to inoculation, and when the medium still remained uncontaminated by intrusive *Bacillus subtilis*, but at some period between that time and the date at which the next cultivation was set—15 days after inoculation—a complete suppression of Comma-Bacilli had taken place, coincidently with abundant growth of intrusive *Bacillus subtilis*.

It now only remains briefly to consider the results of the entire series of experiments. These appear clearly to warrant the following conclusions:—

1. The milk which is in ordinary use in Calcutta normally contains a large number of schizomycete organisms, and in many instances an excessive number of them.

2 The number of distinct species which are normally present is, however, very limited.

3. All of these, save *Bacillus subtilis*, are destroyed by boiling the fluid for a very short time.

4. A certain, but apparently usually only a small number of spores of *Bacillus subtilis* is, however, normally present, and these serve to give rise to an excessive development of that species in the boiled milk.

5. Acid fermentation and coagulation normally occur very rapidly in ordinary specimens of milk.

6. These phenomena are associated with processes of rapid growth and multiplication of the schizomycete organisms which are destroyed by simple boiling of the fluid, and, therefore, in many cases fail to manifest themselves in boiled specimens.

7. In certain cases, however, boiling, while demonstrably destroying the common lacteal schizomycetes, with the exception of *Bacillus subtilis*, fails to prevent acid fermentation and coagulation.

8. This is related to the particular stratum of any sample of milk from which the specimens have been obtained—specimens derived from the upper strata having a much greater tendency to undergo acid fermentation and coagulation than those derived from the lower strata.

9. Cultivations fail to show any differences in the nature of the schizomycetes present in the two areas, and show no important difference in regard to their numbers.

10. The phenomenon must, thus, apparently be explained either as due to a special accumulation of the ferment, elaborated in connection with the growth of the schizomycete organisms, in the upper portions of the fluid, or, more probably, as the result of special accumulation there of those constituents in the milk which are the subjects of the fermentive change which leads to the alteration in reaction and coagulation.

11. Coagulative change ultimately occurs in specimens of milk which have been deprived of all their living schizomycete contents but *Bacillus subtilis.*

12. This, however, differs in nature from the common coagulation of unboiled specimens in occurring apart from the development of acidity to any considerable extent, or as a constant event, in the fluid, and in the character of the resultant coagula, which form a finely pulverulent deposit in place of the large dense masses occurring in the case of common coagulation.

13. The measures which suffice to secure true sterilisation in milk differ somewhat in different instances, due to differences present in the condition of the Bacilli at the time of their application—measures serving to secure sterilisation where no spores are present being naturally not necessarily capable of producing the same effect where spores exist.

14. Complete sterilisation can be certainly arrived at by means of subjecting the milk to the temperature of boiling water for some hours, and after this the fluid remains practically permanently unaltered save in undergoing a gradual evaporative diminution in bulk.

15. The milk in ordinary use in the bazaars and European houses in Calcutta is not a favourable medium for the multiplication or even continued existence of Comma-Bacilli.

16. Their introduction does not interfere with the normal processes of excessive multiplication of the common lacteal schizomycetes and the acid fermentation accompanying it, and, as the latter advances, the Comma-Bacilli rapidly cease to multiply and die out, so that, under normal circumstances, the medium is free of living specimens of them within the course of twenty-four hours.

17. Milk, however, which has been exposed to boiling for a short time becomes a medium in which, for some time, at all events, an excessive multiplication of Comma-Bacilli certainly follows their access.

18. The presence of the Comma-Bacilli in such cases seems to exert a temporary repressive influence on the development of the normal content of *Bacillus subtilis.*

19. The repression is, however, only temporary, and *Bacillus subtilis* again becomes very abundant.

20. This rejuvenescence of *Bacillus subtilis* is sometimes associated with marked decrease in the activity of multiplication of the Comma-Bacilli, or even with their complete suppression, but, in other cases the two species may persist in large numbers in association in the same medium for a period of several weeks' duration.

21. Sterilised milk affords an even more favourable nidus for the growth and multiplication of Comma-Bacilli than boiled milk does, apparently due to the protection which it provides against a struggle for existence.

These facts, beyond their purely scientific interest, appear to have an important bearing practically. They seem to indicate that the ordinary milk supply of the bazaars of Calcutta, and presumably of India generally, furnishes a medium which, from the high degree to which it is normally contaminated by schizomycetes, whose growth is associated with processes of acid fermentation is not at all likely to be favourable to the development or continued existence of those forms which require an alkaline or neutral environment. The very contamination of the fluid comes to render it an unfavourable medium for organisms which, were it pure, would find conditions extremely favourable to their growth and multiplication in it. But, if this be so, unless it were clearly proven that the normal schizomycete population of the milk contains species which are capable, directly or indirectly, of giving rise to disease when they come into relation to the human organism, it can only be regarded as a protective arrangement tending to render the medium unfavourable to the development of other and possibly noxious organisms. It is no doubt unpleasant to realise that the milk supply in India is so highly impure as it unequivocally is, but it does not follow that the contamination is an unmixed evil. For example, if Comma-Bacilli really be the specific cause of cholera, there can be no doubt that the common impurities in milk must tend to render the latter an unfavourable vehicle for the communication of the cause of the disease, and as it is one to which there can be no doubt that Comma-Bacilli are very likely to gain access in any locality in which the disease prevails, those who see in them the essential cause of the disease must regard the normal contamination, or, in other words, the normal schizomycete population of the milk, as playing a beneficent part. The evidence, in so far as it goes, seems to indicate that the ordinary milk supply in this country is an unfavourable medium for schizomycete organisms requiring alkalinity or neutrality in their environment in order to their successful propagation and continued existence apart from the presence of spores, and, this being so, all those who ascribe the development of any disease to the influence of pathogenic schizomycetes may fairly be called on to demonstrate that these are capable of surviving the conditions to which they must normally be exposed on gaining access to milk, ere blaming the latter as a means of communicating the disease. In any case it must be a comfort to realise that if the population of India be condemned to the use of impure milk, the very contamination is likely to act more or less in protective fashion, and is normally due to the presence of organisms which, with the exception of the harmless *Bacillus subtilis*, may be completely destroyed by simple boiling.

Description of the Plate illustrating the Paper.

The negatives from which the Photographs in the Plate were derived show the appearances presented by cultivations of milk under a magnifying power of a little over 11 diameters.

The figures on the left side of the Plate show the numbers of colonies of common lacteal Schizomycetes in cultivations of milk in the state in which it was obtained; those on the right side, the numbers of colonies of *Bacillus subtilis* occurring in cultivations of milk which had been boiled.

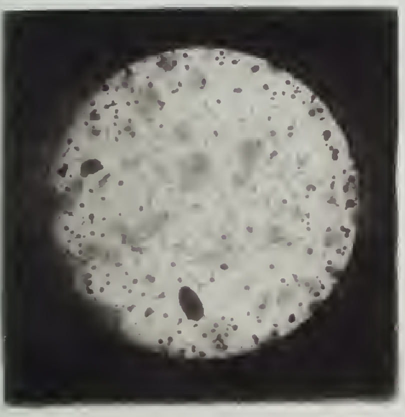
Figures 1 and 5 are derived from cultivations of the same sample of milk before and after boiling, all the colonies in figure 5 consisting of *Bacillus subtilis.*

Figures 3 and 7 correspond in similar fashion.

Figures 3 and 4 are again from a single sample of milk, which was that which had been procured under special precautions. They show the relative numbers of colonies present in a cultivation set immediately after the milk was obtained, and in a second one of a like bulk of the fluid set 24 hours later, and indicate the excessive multiplication of Schizomycetes which had occurred during the interval between the two cultivations.

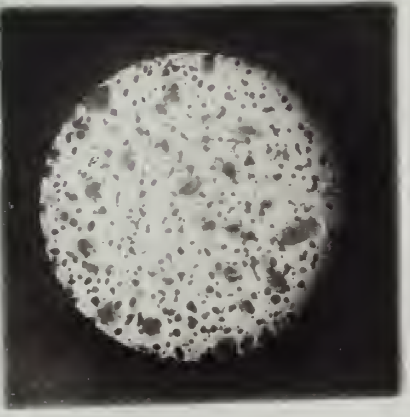
Figure 8 shows the abundance of colonies of *Bacillus subtilis* which developed in a cultivation of milk which had been twice boiled.

1



2

3



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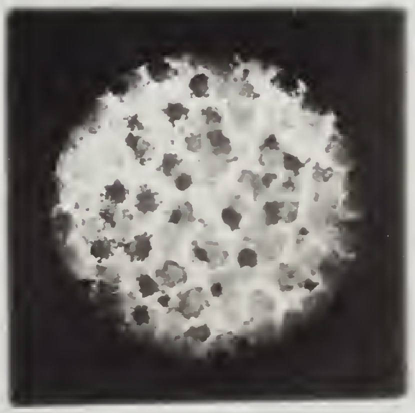
5



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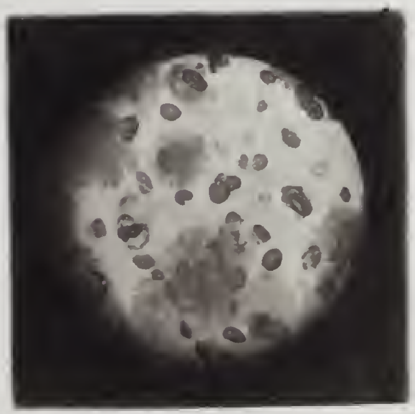
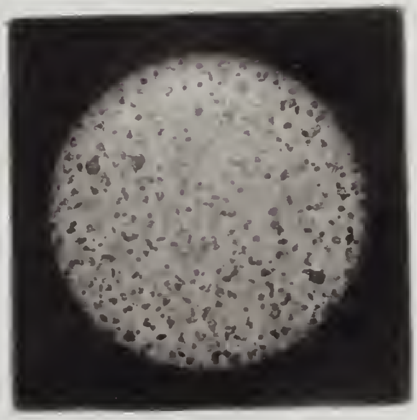


7



8

[illegible] OF UNBOILED AND BOILED SAMPLES OF MILK IN CALCUTTA

Survey of India Offices, Calcutta [illegible]

On the pathological histology of Rhinoscleroma.

BY

SURGEON-MAJOR G. BOMFORD, M.D.,

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An opportunity has occurred during the past year of examining microscopically the affected tissues in five cases of this rare disease. One of them was under the care of Surgeon-Major Raye at the Medical College, Calcutta, and the other four were treated at Indore, Central India, by Surgeon-Major Keegan, the Residency Surgeon. The latter officer has published an account of his cases in the *Indian Medical Gazette* for January 1889, and it is sufficient therefore for the present purpose to state that the diagnosis appears to be unquestionable, as the characteristic clinical phenomena were well marked in all the cases. Mr. Jonathan Hutchinson, on receipt of some sketches of the cases sent to him by Dr. Keegan, writes, "I shall put the sketches in the Museum of the College of Surgeons. They appear to me to be good examples of the disease."

The first specimen received for examination was a portion from the upper lip of Dr. Keegan's first case, preserved in alcohol. That some change had occurred in the tissue was at once evident, for it had a waxy consistence and was wanting in the leathery toughness and tendency to creak under the knife that characterises healthy skin when so preserved. Over the greater part of the surface the epithelium was not obviously changed in its details, but appeared as a whole to be thinned and flattened out, with few and shallow interpapillary processes and scanty interstitial cement substance. There were occasionally, however, distinct projections of the epithelium downwards into the true skin, but this condition was not nearly so frequent nor so pronounced as it often is in Delhi Boil, Elephantiasis, Syphilitic and other inflammatory growths in the skin. In some places also the epithelium had been entirely destroyed by ulceration. There was nothing to be seen at all resembling epithelial nests. The essential change had evidently occurred in the true skin, which was profusely infiltrated with cells of rather a peculiar character and converted into a dense, finely granular, but semi-transparent kind of fibrous tissue. The whole had rather the appearance of fibro-elastic cartilage, and was traversed by dilated vessels, of which the walls were in many cases thickened and hyaline, while the lumen was often packed with leucocytes. The cells were particularly numerous and thickly clustered round the vessels, for which they formed a dense sheath, and, though the majority of them were of small size, $\frac{1}{2000}$th of an inch in diameter, they had an endothelioid appearance, being made up of a fair-sized plum-looking nucleus, attached to a highly transparent oval or circular, apparently flattened, body or plate. Some were, however, of larger size, and a few exceeded $\frac{1}{1000}$th inch in diameter, but these were generally more opaque, and the body of the cell was in them either finely granular or broken up into coarse, angular segments, while the nucleus was disc-shaped and pushed to one side. Many of the smaller cells had a double nucleus, but there were no multi-nucleated giant or myeloid cells. The fibrous material was in part true fibrous tissue, the remains probably of the normal constituent of the skin, but the mass of it appeared to be composed of very much extended hyaline plates, firmly pressed together, except when they were separated by the cells, and devoid of the elongated nuclei and the lustrous transparency of true fibrous tissue. Besides the above, there were in places islands of still smaller cells, granulation cells, in which the nucleus was either vesicular and filled almost the whole cell, or else was broken up into three or four ovoid shrunken fragments, as is often seen in pus-corpuscles.

Subsequently, further specimens were received, including portions of tissue scooped out of the nostril of all five cases, and these differed considerably, at first sight, from the first specimen, as the whole tissue was infiltrated with the small granulation cells, and it was only in places that the endothelial appearance of the cells and the peculiar fibrous tissue could be distinguished. Large areas of every section made from these specimens were so entirely composed of undifferentiated cells that it could be readily understood how the disease has by some pathologists been classified as a sarcoma. The first specimen from the lip would appear to be an example of the more mature growth in a situation where it has been bound down and restricted by the density of the normal tissues, while in all the other specimens we have to do with tne free ebullition of the growth on the yielding surface of the interior of the nostril. In all the specimens there could, however, be no doubt of the ultimate tendency to the formation of the peculiar endothelioid cells and pseudo-fibrous tissue, and the large form of the cell was invariably present. Several of the specimens from the nostril included portions of cartilage which did not appear to be altered in any way, and in all of them there were numerous cells of large size, with a faintly staining nucleus and very distinct, coarse reticulum throughout the whole of the mass of the cell. These had much the appearance of siliceous cells, and are believed to be the result of retention of the siliceous secretion from occlusion of the siliceous ducts.

The above may be considered a fairly complete description of sections of the growth, stained with carmine or hæmatoxylin, but when aniline stains are used two other forms of cell become very conspicuous, especially with methyl-

or gentian violet. The first are remarkably large plasma cells, protoplasmic bodies with a clear, comparatively transparent central region, which includes the nucleus and sometimes a vacuole, surrounded by a thick coating of coarse granules, so that when the cell is seen in a contracted state and deeply stained with violet, it appears as an inky-black granular mass. These plasma cells are particularly noticeable in sections treated by long immersion in methyl-violet and subsequent washing out with alcohol alone; but, on the other hand, if treated by the Gram process with iodine they lose the stain almost completely. With safranin their granular coat stains of a reddish-brown colour, which again renders them very distinct from the surrounding cells. The unequal size of the granules in these cells, their evident density, and the above-mentioned fact that they lose their stain under iodine treatment, is sufficient to prevent them being mistaken for bacteria. Then, in the second place, there were to be seen in all the specimens numerous examples of the hyaline, or colloid, globular bodies described by Cornil and other writers as characteristic of rhinoscleroma. There are bodies composed of one or more, sometimes numerous, perfectly transparent non-refracting globules, associated in most, and possibly in all, cases with a nucleus, and held together by a thin membrane, which is sometimes very indistinct and sometimes certainly appears to be wanting entirely. The globules are not stained at all by carmine (when pure and free from aniline adulteration, which is not always the case) or by hæmatoxylin, but take a yellow stain with picro-carmine, a rather brownish red, but deep, stain with safranin, and, last of all, a very deep stain with any of the aniline violets. They are most effectively shown by staining the sections in either gentian or methyl violet, the former by preference, and then washing out in iodine solution in the manner known as the Gram process. By this process the globules remain deeply stained, when everything else in the section has been washed clean; and the iodine must have the effect of fixing the stain in them, for, if the sections are washed in alcohol alone, without first passing through iodine solution, the hyaline globules are apt to part with the stain in great measure. If, therefore, of two sections of the same tissue, one is washed in alcohol alone and the other "grammed" with iodine, the first will show the plasma cells as above described, while the second will show only the hyaline globules. It is remarkable though that in almost all sections a very careful search will detect some hyaline globules free from stain. With safranin many more of the globules avoid taking the stain than with violet dyes, so that if two sections are taken from a specimen and one is stained with safranin and the other with gentian-violet, on the Gram principle, the latter will always appear at first sight to contain more of the hyaline bodies than the former, the unstained globules escaping detection, except under a very careful examination of the slide. The number of distinct globules associated in each body varies enormously. There may be a solitary globule which is then generally of comparatively large size (as much as $\frac{1}{1000}$th of an inch) with its nucleus flattened out like a half moon on one side; or there may be two globules of equal or unequal size, and the nucleus pressed between them into a triangular or cocked-hat shape; and so on until we come to groups of 30 or 40, which are then almost always of small size, very little, if at all, larger than a blood corpuscle, and either adhere closely together round a central nucleus to form a mulberry mass or are only loosely connected, and it is in this last case sometimes impossible to demonstrate either a nucleus, or any limiting membrane. Occasionally the component hyaline particles are not globular, but wedge-shaped or polyhedral, which is evidently the result in many cases of mutual pressure, but not always, for a very remarkable form is sometimes met with, *viz.* a solitary hexagon of large size, surrounded by a thin membrane and provided with a nucleus on one side, just as in the case of the largest globular forms.

The nature of these hyaline bodies is a mystery, but they are certainly not peculiar to rhinoscleroma. Identically the same bodies were described in 1885 by Surgeon-Major D. D. Cunningham in Part I of these Memoirs, as occurring in Delhi Boil. He was at that time inclined to look upon them as organisms of some kind, and this view was confirmed by the remarkable discovery that bodies of similar form and showing the same re-actions with aniline violets, are found in certain encysted amæboid organisms; these being possibly the same as the globular bodies found by Nothnagel in monads, *vide* Ziegler's Pathology, para. 479.

It has recently, however, been found by the writer that precisely similar forms are constantly present in Elephantiasis arabum, in small number and of small size it is true in the ordinary fibrous condition of the disease; but abundant and of every variety and form, just as in rhinoscleroma, in some unusually cellular specimens of elephantiasis excised from the female genital organs at the Ripon Hospital, Simla, where this disease is frequently met with in women. They were also undeniably present, although they were not very conspicuous, in two distinct cases of carcinomatous tumour from the same hospital, one of the face, the other of the breast; and Dr. Gibbons, of the Medical College, Calcutta, has found them in a papilloma at the orifice of the urethra.

Dittrich also, of Prague, who calls them "Mikuliczsche Zellen" states that they are found, not only in rhinoscleroma and oriental sore, but also in leprosy and glanders. Under these circumstances the view that they are organisms of any kind seems hardly tenable, as it is difficult to imagine a special organism inhabiting so many different kinds of growth. Another view, upheld particularly by Dittrich, that these bodies are the result of the action of bacteria upon the cells of the tissue, cannot be said to have ever had much foundation, for nobody seems ever to have seen bacteria actually within the hyaline masses, and it is effectually disposed of by the fact, to be presently discussed more fully, that there were no bacteria present in any of these cases of rhinoscleroma, and that bacteria were also wanting in the Delhi Boil examined by Dr. Cunningham in the above mentioned carcinomata, and the papilloma, as well as in most of the cases of elephantiasis examined by the writer at Simla. That they are the result of some colloid or hyaline change in cells, not necessarily always of the same variety of cell, and perhaps in some cases even of the blood corpuscles seems probable enough; but as yet no thoroughly satisfactory transitional form can be discovered. Some of the large cells, with excentric nucleus and granular or fragmentary contents, would, as far as mere form goes, answer well enough as a transition between the endothelioid cells and the hyaline globules; and some of them particularly, in which the contained fragments are wedge-shaped or polyhedral, are almost exactly similar in form to some of the hyaline bodies, but they show no special tendency to stain, with aniline violets, and, on the contrary, stain, often deeply, with carmine.

The number of the hyaline bodies varied considerably in the different specimens. In Dr. Raye's case there were very few, while in one of the Indore cases they were not only very numerous, ten or even more in one field of the microscope, but of remarkably large size. A large proportion of them are situated just below the epithelium in the papillary region of the true skin, but they are never apparently included in the epithelial layer.

There remains to be discussed only the question of the presence or otherwise in these cases of the so-called rhinoscleroma bacillus or any other schizomycete. Investigations on this point were carried on independently by Surgeon-Major D. D. Cunningham, Surgeon Gibbons, and the writer, with an entirely barren result in every instance. The most approved methods of staining with gentian and methyl violets, with long immersion in the staining fluids, and washing out with iodine solution, or with alcohol alone, as recommended by Cornil, all failed to demonstrate anything which could be considered a bacillus. The conclusion therefore is inevitable that we have to do here with five undoubted cases of Rhinoscleroma in which the disease is not even associated with a bacillus, and accordingly the claim of the form hitherto described as occurring constantly in Rhinoscleroma to be the cause of the disease may be safely dismissed. It is satisfactory to learn that a similar negative conclusion on this point has been arrived at by a distinguished bacteriologist in London, for Mr. Jonathan Hutchinson, in the same letter to Dr. Keegan that was quoted above, says—, "Dr. Payne tells me that he has examined your specimens and not found the parasite." It is unfortunately not known to the writer where Dr. Payne has published this interesting fact.

CALCUTTA:
June 1889.

A. List of Laccadive Plants.

BY

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West of the Malabar coast, between Lat. 10° and 14° N. and Long. 71° 40′ and 74° E., and distant 120—180 miles from the mainland of India, lie 14 small islands, known as the Laccadives. The islands are of coral formation, and with hardly an exception each consists of only some portion of an atoll ring, the remainder of which may be uncovered at low tide, but is more usually merely indicated by a ring of breakers and a circle of shoal water round a somewhat deeper lagoon-space. These atolls were formerly supposed to be situated on a ridge that further to the south accommodated in succession the Maldive and the Chagos Archipelagos, this ridge being about 300 fathoms from the surface and the trough between it and the Indian coast exceeding 900 fathoms in depth. But they are now known to "form a chain of peaks rising from a bed of 1,100 fathoms, or are in themselves 6,600 feet above the bottom."[1] Three of the atolls are merely open reefs; the others form 11 islands, of which 9 are inhabited; none of them are elevated more than 20 feet above the level of the sea. Whether the atolls be rising or subsiding is an unsettled point. Local traditions speak of the west coast of India as being gradually upraised; on the other hand, physiographers favour the view that subsidence is taking place in this region.[2] And there does not appear to be any published account of the corals that compose the atolls, or to have been any attempt made to deduce therefrom the probable age of the group. But whatever their antiquity, it is probable that the appearance of these islands was long subsequent to the establishment of something like the present physical configuration of the adjacent mainland. At the same time they have a human history of over 500 years, for it was about 1500 A. D. that the inhabitants were converted to Mahomedanism, and the Sub-Hinduism indicated by deep-rooted caste prejudices, which cannot here be attributed to contact with Hindu neighbours, but which is nevertheless strongly marked, appears to point to the fact that the people were present in the islands as Hindus before they became Musalmans.

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[1] Carpenter: Administration Report of the Marine Survey of India for the year 1888-89.

[2] Ansted: Physical Geography, ed. ii, 70; Medlicot and Blanford, Geology of India, Part i, 376. Subsidence is in both works deduced from the existence of coral islands. This line of reasoning, however, which appeared when employed by these authors to be direct, has, owing to the vexed state of specialist opinion that has lately arisen concerning coral formation, been converted into a closed curve.

These two considerations have to be borne in mind in investigating the problem with which this paper proposes to deal—the nature and the probable origin of the flora of the archipelago. And to them has to be added a third; it does not necessarily follow from their present condition that the islands now inhabited have always been so throughout the human history of the archipelago, or that those now uninhabited have always been without people. For the wearing action of the ocean on the islands must at all times be considerable, and their elevation above sea-level is so slight that severe cyclones passing along the Malabar coasts give rise to storm-waves high enough to cover them. In 1847 such a wave destroyed 1,000 of the whole population, and in 1867 a similar wave drowned half that number in Minikoi alone.[1] And evidence of other kinds is not altogether wanting. When Mr. A. O. Hume visited the archipelago in 1875 he found the people of Kiltan cultivating the plantain and various other garden plants and exhibiting all the appearances of being comfortably off.[2] In 1881 the population of this island was 790.[3] But now they are miserably ill-off, and when Dr. Alcock visited the islands in 1889[4] possessed few of the comforts seen by Mr. Hume, *e.g.* they did not cultivate the plantain, while they were actually doing a little fishing; the account of the native official on the island at the same time was that the population had fallen to 450, or thereabouts. Again, in Betrapar, of which there is no record that it ever was inhabited, Mr. Hume collected certain species of plants that could hardly have been introduced, except by the agency of man.

Our knowledge of the flora of the archipelago is probably by no means complete. Mr. Hume collected in the islands that he visited, and references to the species he saw are to be found in the paper already referred to. The collection itself he kindly presented to the Calcutta Herbarium, where it is now preserved. And Dr. Alcock made a small collection in two of the islands, which he also has kindly sent to Calcutta. Nothing has been received from the archipelago during the interval between 1875 and 1889. Mr. Hume did not visit Anderut, one of the islands in which Dr. Alcock collected; Dr. Alcock had the opportunity of visiting only one of the islands examined by Mr. Hume. While, therefore, there are a number of species mentioned by Mr. Hume that Dr. Alcock did not see, there are, on the other hand, some in Dr. Alcock's collection that were not met with by Mr. Hume. Mr. Hume's collection has, therefore, been re-examined, and his notes studied along with the notes and specimens of Dr. Alcock, and a list drawn up which includes the plants observed or collected by both these gentlemen. In every case exact localities are given, and the mark of verification (!) added when the specimen is present at Calcutta. The list is probably by no means complete, since of the 80 species that it includes Dr. Alcock only reports 38, while of these 38 Mr. Hume omits 16. At the same time it probably represents fairly accurately the nature of the flora. The objects of its publication are, first, to afford a means of comparison between the present flora and the flora that may be observed after some considerable interval, and second, to provide a basis for the determination of the sources from which it has been derived and the means by which its constituents have been conveyed to the islands—

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[1] Hunter: Gazetteer of India, ed. ii, vol. viii, p. 396.

[2] Hume: The Laccadives and the West Coast; Stray Feathers (1876), vol. iv, pp. 413 *et seq.*:—a charming account of the voyage during which this visit was made.

[3] Hunter, l. c., page 392.

[4] Notes made by Dr. Alcock during 1888-89 in a voyage of H. M.'s *Investigator.*

List of the Plants.

[Reference is made mainly to descriptions in Dr. Roxburgh's *Flora Indica* and in Sir J. D. Hooker's *Flora of British India.*]

Capparideæ—

1. Cleome viscosa *L.; Roxb., Flor. Ind.* iii, 128; *Hook. f., Flor. Brit. Ind.* i, 170.
   Ameni, (Hume !); Anderut, (Alcock !).

Guttiferæ—

2. Calophyllum inophyllum *L.; Roxb., Flor. Ind.* ii, 606; *Hook. f., Flor. Brit. Ind.* i, 273.
   Ameni, (Hume); cultivated. The Poon-spar tree or Alexandrian Laurel.

Malvaceæ—

3. Sida humilis *Willd.; Roxb., Flor. Ind.* iii, 171; *Hook. f., Flor. Brit. Ind.* i, 322.
   Cardamum, (Hume !).
4. Abutilon indicum, *G. Don.; Hook. f., Flor. Brit. Ind.* i, 326. Sida indica *Roxb., Flor. Ind.* iii, 179.
   Cardamum, (Hume !).
5. Thespesia populnea *Corr.; Hook. f., Flor. Brit. Ind.* i, 345. Hibiscus populneus, *Roxb., Flor. Ind.* iii, 190.
   Anderut, (Alcock !); planted.

Rutaceæ—

6. Citrus medica *L.* VAR acida *Brandis; Hook. f., Flor. Brit. Ind.* i, 515. C. acida *Roxb., Flor. Ind.* iii, 390.
   Ameni, (Hume); cultivated. Dr. Alcock does not note having seen this grown in Anderut.

Simarubeæ—

7. Suriana maritima *L.; Hook. f., Flor. Brit. Ind.* i, 522.
   Betrapar, (Hume !); Bungaroo, (Hume !).

Ampelidæ—

8. Vitis carnosa *Wall.; Hook. f., Flor. Brit. Ind.* i, 654. Cissus carnosa *Roxb., Flor. Ind.* i, 409.
   Kiltan, (Alcock !); in great profusion.

Sapindaceæ—

9. Cardiospermum Halicacabum *L.; Roxb., Flor. Ind.* ii, 292; *Hook. f., Flor. BritInd.* i, 670.
   Ameni, (Hume !).

Moringeæ—

10. Moringa pterygosperma *Gaertn.*; *Hook. f., Flor. Brit. Ind.* ii, 45. Hyperanthera Moringa *Roxb., Flor. Ind.* ii, 368.
Kiltan, (Hume); cultivated. The Horse-radish tree.

Leguminosæ—

11. Crotalaria verrucosa *L.*; *Roxb., Flor. Ind.* iii, 273; *Hook. f., Flor. Brit. Ind.* ii, 77.
Ameni, (Hume !).

12. Clitoria Ternatea *L.*; *Roxb., Flor. Ind.* iii, 321; *Hook. f., Flor. Brit Ind.* ii, 208.
Ameni, (Hume); cultivated.

13. Mucuna capitata *W. & A.*; *Hook. f., Flor. Brit. Ind.* ii, *187*. Carpopogon capitatum *Roxb., Flor. Ind.* iii, 284.
Ameni, (Hume !).

14. Cæsalpinia Bonducella *Flem.*; *Roxb., Flor. Ind.* ii, 357; *Hook. f., Flor Brit. Ind.* ii, 254.
Bungaroo, (Hume !); forming a dense low jungle.

15. Tamarindus indica *L.*; *Roxb., Flor. Ind.* iii, 215; *Hook. f., Flor. Brit. Ind.* ii, 273.
Ameni, (Hume); cultivated. The Tamarind.

Lythraceæ—

16. Punica Granatum *L.*; *Roxb., Flor. Ind.* ii, 499; *Hook. f., Flor. Brit. Ind.* ii, 581.
Ameni, (Hume); cultivated. The Pomegranate.

Passifloreæ—

17. Carica Papaya *L.*; *Roxb., Flor. Ind.* iii, 824; *Hook. f., Flor. Brit. Ind.* ii, 599.
Kiltan, (Hume); Anderut, (Alcock); cultivated. The Papaw.

Rubiaceæ—

18. Oldenlandia diffusa *Roxb., Flor. Ind.* i, 423; *Hook. f., Flor. Brit. Ind.* iii, 65.
Anderut, (Alcock !).

19. Ixora coccinea *L.*; *Hook. f., Flor. Brit. Ind.* iii, 145. I. Bandhuca *Roxb., Flor. Ind.* i, 376.
Cardamum, (Hume !); Anderut, (Alcock). Sir J. D. Hooker shews that *I. Bandhuca* is really conspecific with *I. coccinea*. There is, however, no possibility of mistaking the one form for the other as they grow, and it must be noted that the plant observed by Mr. Hume and Dr. Alcock was not typical *I. coccinea* but the form described by Dr. Roxburgh as *I. Bandhuca*.

20. Morinda citrifolia *L.* VAR. bracteata *Hook. f., Flor. Brit. Ind.* iii, 156. M. bracteata *Roxb., Flor. Ind.* i, 544.
Bungaroo, (Hume !).

21. Guettarda speciosa *L.*; *Roxb., Flor. Ind.* i, 686; *Hook. f., Flor. Brit. Ind.* iii, 126.
Cardamum, (Hume !).

Compositæ—

22. Vernonia cinerea *Less.; Hook. f., Flor. Brit. Ind.* iii, 233. Serratula cinerea *Roxb., Flor. Ind.* iii, 406.
Ameni, (Hume !) ; Anderut, (Alcock !).

23. Ageratum conyzoides *L.; Hook. f., Flor. Brit. Ind.* iii, 243. A. cordifolium *Roxb., Flor. Ind.* iii, 415.
Ameni, (Hume !) ; Anderut, (Alcock !).

24. Wedelia calendulacea *Less.; Hook. f., Flor. Brit. Ind.* iii, 306. Verbesina calendulacea *Roxb., Flor. Ind.* iii, 440.
Anderut, (Alcock !).

25. Wedelia biflora *DC.; Hook. f., Flor. Brit. Ind.* iii, 306. Verbesina biflora *Roxb., Flor. Ind.* iii, 440.
Cardamum, (Hume !) ; Anderut, (Alcock !).

26. Crepis acaulis *Hook. f., Flor. Brit. Ind.* iii, 396. Prenanthes acaulis *Roxb. Flor. Ind.* iii., 403.
Kiltan, (Alcock !).

27. Launea pinnatifida *Cass.; Hook. f., Flor. Brit. Ind.* iii, 416.
Betrapar, (Hume !). This is probably what Dr. Roxburgh means by his *Prenanthes asplenifolia* (*Flor. Ind.* iii, 404), at least partly and as regards the plant "common on the sand-hills near the sea along the Coromandel Coast" (p. 405).

Goodenovieæ—

28. Scaevola Koenigii *Vahl; Hook. f., Flor. Brit. Ind.* iii, 421. S. Taccada *Roxb., Flor. Ind.* i, 527.
Betrapar, (Hume !) ; Kiltan, (Alcock !).

Plumbagineæ—

29. Plumbago zeylanica, *L.; Roxb., Flor. Ind.* i, 462. ; *Hook. f., Flor. Brit. Ind.* iii, 480.
Ameni, (Hume !).

Asclepiadeæ—

30. Calotropis gigantea *R. Br.; Hook. f., Flor. Brit. Ind.* iv, 17. Asclepias gigantea *Roxb., Flor. Ind.* ii, 30.
Cardamum, (Hume !).

31. Cynanchum alatum *W. & A.; Hook. f., Flor. Brit. Ind.* iv, 23.
Kiltan, (Alcock !). The Laccadive plant agrees in every detail with Nilghiri specimens.

32. Tylophora asthmatica *W. & A.; Hook. f., Flor. Brit. Ind.* iv, 45. Asclepias asthmatica, *Roxb., Flor. Ind.* ii, 33.
Cardamum, (Hume !) ; Anderut, (Alcock !).

Boragineæ—

33. Tournefortia argentea *L. f.; Hook. f., Flor. Brit. Ind.* iv, 145.
Betrapar, (Hume !) ; Kiltan, (Alcock !).

Convolvulaceæ—

34. Ipomœa grandiflora *Lamk.; Hook. f., Flor. Brit. Ind.* iv, 198.

Convolvulaceæ—*contd.*

Betrapar, (Hume !). This is not the same plant as Dr. Roxburgh's *I. grandiflora*, which is a variety of *I. bona-nox* of Linnæus. *I. bona-nox* is the true Moon-flower, a plant originally introduced from America. *I. grandiflora*, on the other hand, is a native of the old world, which appears in America only as an introduced plant.

35. Ipomœa Batatas *Lamk.; Hook. f., Flor. Brit. Ind.* iv, 202. Convolvulus Batatas *Roxb., Flor. Ind.* i, 483.
Ameni, (Hume); Anderut, (Alcock); cultivated. The Sweet Potato.

36. Ipomœa biloba *Forsk.; Hook. f., Flor. Brit. Ind.* iv, 212. Convolvulus Pes-capræ *Roxb., Flor. Ind.* i, 486.
Betrapar, (Hume !).

Solanaceæ—

37. Physalis minima *L.; Roxb., Flor. Ind.* i, 563; *Hook. f., Flor. Brit. Ind.* iv, 238.
Anderut, (Alcock !).

38. Physalis peruviana *L.; Roxb., Flor. Ind.* i, 562; *Hook. f., Flor. Brit. Ind.* iv, 238.
Ameni, (Hume); Bungaroo, (Hume !); cultivated. The Winter-cherry or Cape-gooseberry.

39. Datura fastuosa *L.; Roxb., Flor. Ind.* i, 561; *Hook. f., Flor. Brit. Ind.* iv. 242.
Ameni, (Hume); Anderut, (Alcock).

Scrophulariaceæ—

40. Herpestis Monniera *H. B. & K.; Hook. f., Flor. Brit. Ind.* iv, 272. Gratiola Monniera *Roxb., Flor. Ind.* i, 141.
Anderut, (Alcock !).

Acanthaceæ—

41. Barleria Prionitis *L.; Roxb., Flor. Ind.* iii, 36; *Hook. f., Flor. Brit. Ind.* iv, 482.
Ameni, (Hume !).

42. Barleria cristata *L.; Roxb., Flor. Ind.* iii, 37; *Hook. f., Flor. Brit. Ind.* iv, 488.
Ameni, (Hume !).

43. Rungia parviflora *Nees* VAR. pectinata *Clarke; Hook. f., Flor. Brit. Ind.* iv, 550. Justicia pectinata *Roxb., Flor. Ind.* i, 133.
Ameni, (Hume !).

44. Peristrophe bicalyculata *Nees; Hook. f., Flor. Brit. Ind.* iv, 554. Justicia bicalyculata *Roxb., Flor. Ind.* i, 126.
Ameni, (Hume !).

Verbenaceæ—

45. Stachytarpheta indica *Vahl.; Hook. f., Flor. Brit. In* iv, 564.
Anderut, (Alcock !).

46. Premna integrifolia *L.; Hook. f., Flor. Brit. Ind.* iv, 574. P. spinosa *Roxb., Flor. Ind.* iii, 77.
Cardamum, (Hume !); forming thickets.

Labiatæ—

47. Leucas aspera *Spr.; Hook. f., Flor. Brit. Ind.* iv, 690. L. esculenta *Roxb., Flor. Ind.* iii, 10.
Ameni, (Hume!); Anderut, (Alcock!).

Nyctagineæ—

48. Boerhaavia repens *L.* VAR. diffusa *Hook. f., Flor. Brit. Ind.* iv, 709. B. diffusa *L., Sp. Pl.* 3. B. procumbens *Roxb., Flor. Ind.* i, 146.
Betrapar, (Hume!); Ameni, (Hume!); Anderut, (Alcock!).

Amarantaceæ—

49. Aerua lanata *Juss.; Hook. f., Flor. Brit. Ind.* iv, 728. Achyranthes lanata *Roxb., Flor. Ind.* i, 676.
Betrapar, (Hume!).

50. Achyranthes aspera *L.; Roxb., Flor. Ind.* i, 672; *Hook. f., Flor. Brit. Ind.* iv, 730.
Betrapar, (Hume!); Bungaroo, (Hume!).

Laurineæ—

51. Hernandia peltata *Meissn.; Hook. f., Flor. Brit. Ind.* v, 188.
Caurati, (Hume!).

Euphorbiaceæ—

52. Euphorbia Atoto *Forst.; Hook. f., Flor. Brit. Ind.* v, 248.
Ameni, (Hume!).

53. Euphorbia pilulifera *L.; Hook. f., Flor. Brit. Ind.* v, 250. E. hirta *Roxb., Flor. Ind.* ii, 472.
Cardamum, (Hume!); Kiltan, (Alcock!); Anderut, (Alcock!).

54. Phyllanthus Emblica *L.; Roxb., Flor. Ind.* iii, 671; *Hook. f., Flor. Brit. Ind.* v, 289.
Ameni, (Hume); cultivated. The Amla.

55. Phyllanthus maderaspatensis *L.; Roxb., Flor. Ind.* iii, 654; *Hook. f., Flor. Brit. Ind.* v, 292.
Ameni, (Hume!); Anderut, (Alcock!).

56. Acalypha indica *L.; Roxb., Flor. Ind.* iii, 675; *Hook. f., Flor. Brit. Ind.* v, 416.
Anderut, (Alcock!).

57. Ricinus communis *L.; Roxb., Flor. Ind.* iii, 689; *Hook. f., Flor. Brit. Ind.* v, 457.
Kiltan, (Hume); Anderut, (Alcock); cultivated. The Castor-oil Plant.

Urticaceæ—

58. Ficus bengalensis *L.; King, Hook. f., Flor. Brit. Ind.* v, 499. F. indica *Roxb., Flor. Ind.* iii, 539.
Ameni, (Hume); planted. The Banyan.

59. Artocarpus incisa *L. f.; Roxb., Flor. Ind.* iii, 527; *Hook. f., Flor. Brit. Ind.* v, 539.
Kiltan, (Hume, Alcock); Ameni, (Hume); Anderut, (Alcock); cultivated. The Bread-fruit Tree.

Scitamineæ—

60. Musa sapientum *L.; Roxb., Flor. Ind.* i, 663.
Kiltan, (Hume); cultivated. The Plantain.

Amaryllidæ—

61. Agave vivipara *L.* A. Cantula *Roxb., Flor Ind.* iii, 167.
Anderut, (Alcock); cultivated. The American Aloe.

Dioscoreæ—

62. Dioscorea sativa *Willd.*
Ameni, (Hume); cultivated. The Yam.

Liliaceæ—

63. Gloriosa superba *L.; Roxb., Flor. Ind.* ii, 143.
Anderut, (Alcock!).

Palmeæ—

64. Areca catechu *L.; Roxb., Flor. Ind.* iii, 615.
Ameni, (Hume); Anderut, (Alcock); cultivated. The Betel Palm.

65. Cocos nucifera *L.; Roxb., Flor. Ind.* iii, 614.
Betrapar, (Hume); Ameni, (Hume); Kiltan, (Hume, Alcock); Anderut, (Alcock). On all the inhabited islands and also on Betrapar, which is not inhabited. The Coco-nut Palm.

Pandaneæ—

66. Pandanus odoratissimus *Willd.; Roxb., Flor. Ind.* iii, 738.
Kiltan, (Hume); Cardamum, (Hume).

Aroideæ—

67. Colocasia antiquorum *Schott.* Arum colocasia *Roxb., Flor. Ind.* iii, 494.
Ameni, (Hume); Anderut, (Alcock); cultivated. The Kachhu or Taro.

Cyperaceæ—

68. Cyperus arenarius *Retz.; Clarke, Cyper. Ind.* 106.
Betrapar, (Hume).

69. Cyperus pennatus *Lamk.; Clarke, Cyper. Ind.* 194.
Bungaroo, (Hume), Anderut, in great profusion, (Alcock!).

Gramineæ—

70. Oplismenus compositus *R. & S.* Panicum lanceolatum *Roxb., Flor. Ind.* i, 294.
Ameni, (Hume!).

71. Setaria verticillata *Beauv.* Panicum verticillatum *Roxb., Flor. Ind.* i, 301.
Bungaroo, (Hume!).

72. Spinifex squarrosus *L.*
Betrapar, (Hume!).

73. Andropogon contortus *L.; Roxb. Flor. Ind.* i, 253.
Kiltan, (Alcock!).

Gramineæ—*continued.*

74. Apluda aristata *L.; Roxb., Flor. Ind.* i, 324.
Cardamum, (Hume!).

75. Cynodon Dactylon *Pers.?* Panicum Dactylon *Roxb., Flor. Ind.* i, 289.
Bungaroo, (Hume). Mr. Hume refers one of the grasses he saw in Bungaroo to this—the common Dubh. He did not collect specimens however.

76. Eleusine ægyptiaca *Pers.; Roxb., Flor. Ind.* i, 344.
Ameni, (Hume!).

77. Eleusine Coracana *Gaertn.; Roxb. Flor. Ind.* i, 342?
Anderut, (Alcock); cultivated. Dr. Alcock saw a small quantity of some cereal crop which he did not recognise under cultivation. This is almost certain to have been the *Rhagi* crop (*Eleusine Coracana*).

78. Lepturus repens *R. Br.*
Bungaroo, (Hume!). This coast-grass Mr. Hume also collected at Vingorla rocks on the western coast of India.

Filices—

79. Nephrodium molle *Desv.; Beddome, Ferns of Brit. Ind.*, 277.
Anderut, (Alcock!).

80. Nephrolepis tuberosa *Presl.* N. cordifolia *Bedd., Ferns, Brit. Ind.* 282.
Anderut, (Alcock!).

Of these 80 species then, 78—or 97½ per cent.—are Phanerogams.

An analysis of the list shows how extensively man has influenced the composition of the flora.

The following 17 species are in the strictest sense cultivated:—

Lime.
Horse-radish tree.
Tamarind.
Pomegranate.
Papaw.
Sweet potato.
Winter-cherry.
Amla (*Phyllanthus Emblica*).
Castor-oil plant.
Bread-fruit tree.
Plantain.
American Aloe.
Yam.
Betel-palm.
Kachhu (*Colocasia*).
The cereal crop (*Rhagi*?).
Coco-nut.

Except the coco-nut palm and the castor-oil plant all of these are manifestly intentionally introduced, and as regards the castor-oil plant, though its seeds have at times been found in ocean drifts, and though it is conceivable that it may have arrived as a weed, the probabilities are greatly in favour of its having been intentionally brought. It is largely cultivated elsewhere in India, and is cultivated by the Laccadive Islanders also. With the coco-nut palm the conditions are different, and the evidence, both intrinsic and extrinsic, indicates that its appearance in the archipelago preceded that of man. Its fruits are highly capable of ocean-distribution and form a constant feature of ocean-drifts on tropical shores; it is one of the first species to appear on newly emerged or unvisited tropical islands; among the Laccadives themselves it occurs on the uninhabited islands, as well as the inhabited. On the other hand, if man did not settle on the archipelago on account of its coco-nut trees, it is difficult to conceive what he went there for at all. The surface is not adequate nor are the conditions favourable for cereal cultivation ; and fishing is exactly the occupation that may be said to be neglected by the Laccadive Islanders. It is true that they may not always have neglected their opportunities of fishing ; but the fact that in islands where they are well-to-do the inhabitants now-a-days do not fish, taken in conjunction with the evidence derived from the facts of coco-nut distribution elsewhere, makes it almost certain that the coco-nut has reached the Laccadives independently of man's agency. Of the 17 cultivated species then, man has certainly introduced 15, and probably 16. But these do not exhaust the number of species intentionally introduced. Three others :—

Banyan,
Ixora coccinea,
Clitoria Ternatea.

are not only under cultivation at present, but cannot have their presence well explained except by assuming that they have been intentionally introduced. The Banyan is not, it is true, a very favourite tree with Musalmans ; still, there is a certain amount of sentiment in connection with it which even the good Musalman does not readily overcome, and which must be unusually strong in a Sub-Hindoo Musalman population, like that of the Laccadives. The *Ixora* grows at the edges of the small patches of cultivation ; and, though it may have been introduced only on account of its ornamental flowers, it must be borne in mind that many natives of India ascribe, and probably with justice, valuable anti-dysenteric qualities to the bark of its roots. It should be noticed too that the Laccadive plant is not *Ixora coccinea* proper, but a form of the species which Dr. Roxburgh considered specifically distinct, and to which he gave the name *I. Bandhuca,* and it is to this form that medicinal virtues are especially attributed. The *Clitoria* is a very favourite creeper in all Indian gardens, and is perhaps grown by the islanders only on account of the beauty of its flowers ; it has, however, a reputation for the cathartic properties of its roots and of its seeds, those of the former being the more powerful.

Besides the castor-oil plant, however, there are 16 other species that it is possible the islanders may have introduced intentionally. These are :—

The Poon-spar tree (*Calophyllum*).
The Portia tree (*Thespesia*).
Mucuna capitata.
Cæsalpinia Bonducella.
Morinda citrifolia.
Plumbago zeylanica.
Calotropis gigantea.
Cynanchum alatum.
Tylophora asthmatica.
Ipomœa grandiflora.
Datura fastuosa.
Stachytarpheta indica.
Premna integrifolia.
Gloriosa superba.
Pandanus odoratissimus.
Cynodon Dactylon (?)

As, however, it is probable that many of these have reached the islands independently of man's agency, while others, though introduced by man, may have been brought unintentionally, it becomes necessary to analyse this list in detail.

The Poon-spar, or Alexandrian Laurel, is (along with *Barringtonia speciosa*) about the earliest species to appear on newly emerged uninhabited coral islets, and *primâ facie* therefore might be looked upon as an ocean-distributed plant. But on closer consideration the case is less clear. The tree is much cultivated in India and is planted by the Laccadive Islanders, as it is by the people on the opposite coast, to provide them with masts and spars. Then, not only is it planted where it occurs, but it does not seemto occur on every island (*e.g.* Dr. Alcock did not observe it on Anderut, which is moreover the island nearest India), and the species that elsewhere is so intimately associated with it (*Barringtonia*), does not appear to have reached the archipelago. So far then as the Laccadives are concerned, it seems necessary to look on the Poon-spar as a species introduced intentionally by man.

The case of the Portia tree (*Thespesia*) is identical. It also is a shore species; its fruits occur in eastern tropical ocean-drifts, and it is widely distributed on eastern tropical shores. But it is, on the other hand, a favourite tree and is much planted, especially in Madras and in Ceylon, both on account of its showy flowers and because of its tough and durable wood used in boatbuilding. It only occurs on one island—that which has most intercourse with the mainland—and it is there distinctly a planted species. Its intentional introduction by man is therefore much more certain than even in the preceding instance.

The common Cowhage (*Mucuna pruriens*), to which *Mucuna capitata* is closely allied, has a very wide distribution, and seeds of various species of *Mucuna* have frequently been found in ocean-drift, and that too in a germinable condition after having traversed wide intervals of sea. There is thus no intrinsic reason why *Mucuna capitata* may not have been carried to the Laccadives by ocean-currents. But, on the other hand, this particular species is in the Laccadives, as it appears to be everywhere else, purely a garden plant; it therefore is almost certainly an intentionally introduced species.

With *Cæsalpinia Bonducella* the case is different. This is a widely distributed tropical plant, whose seeds occur in many ocean-drifts; it is beyond doubt an ocean-distributed plant elsewhere; in the islands where it occurs it forms a dense jungle. So, while it is just within the bounds of possibility that it may have been introduced on account of the antiperiodic properties of its bitter seeds, the probability is that it has been introduced by ocean-currents.

The evidence as to *Morinda* is not altogether clear. True *M. citrifolia* is a wide-spread species that probably only occurs in India as a cultivated plant, and is very generally planted throughout the Malay Archipelago, North Australia, Polynesia and Africa,[1] mainly for the dye-stuff yielded by its roots. But the Laccadive plant is not true *M. citrifolia*, but that variety which Dr. Roxburgh considered a distinct species and named *M. bracteata*. This plant is confined to India, Ceylon, and the Andamans, is perhaps oftener wild than cultivated, and was, where Mr. Hume found it, certainly wild. Unless then it be assumed that this plant has here escaped from cultivation, and that its cultivation has been at the same time given up, the species must be looked on as an example of a sea-introduced plant. Mr. Hemsley ("*Challenger*" Reports; Botany, Introd., p. 43) contends for an ocean-distribution for true *M. citrifolia*, and the evidence from this variety certainly supports his view.

Plumbago zeylanica is a common plant in Indian gardens, and, besides being grown for its appearance, is used in Indian domestic medicine in skin diseases. It has therefore probably been introduced intentionally. At the same time it is a plant that readily throws itself out of cultivation, and it may have reached the Laccadives only as a weed.

Calotropis gigantea may have been intentionally introduced. Its milky juice is medicinal, and it yields an excellent fibre used along the western coast of India. At the same time it is a common road-side weed everywhere on the mainland, and it may be as a weed that it has reached the islands, while there is nothing to have prevented its seeds from having been brought from the mainland by winds. Then both *Cynanchum* and *Tylophora* may have been introduced on account of their juice, since they too are Milk-weeds; it is, however, far more probable that they have reached the islands with the assistance of wind.

The large-flowered *Ipomœa* may have been intentionally introduced, or more probably perhaps, have arrived as a weed. At the same time the seeds of several species of *Ipomœa* occur frequently in ocean-drifts, and some are characterized by the readiness with which they become distributed by ocean-currents. To strengthen this possibility we have here the fact that Mr. Hume found the species in Betrapar, which is one of the uninhabited islands. On the whole then this species is more probably sea-distributed, though the mere fact that it occurs where there are no inhabitants does not render the evidence conclusive; since the island is visited by the inhabitants of the other islands of the group, and since it does not follow, because Betrapar has no permanent inhabitants now, that this has been always the case.

The *Datura* may have been introduced purposely, for it is not infrequently deliberately cultivated by evil-disposed people. It is, however, a common Indian weed, and has probably been only unintentionally introduced.

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[1] Mr. Hemsley ("*Challenger*" Reports; Botany, vol. i, part iii, p. 109) gives America also.

Stachytarpheta indica is a common and favourite flower in Indian gardens, and may have been intentionally introduced. But it is also (like *Plumbago*) a plant that readily throws itself out of cultivation, and is nearly as likely to have been unintentionally conveyed.

Premna integrifolia may possibly have been introduced because of its medicinal properties. It has, however, more probably arrived independently of man. Mr. Hume found it wild, and in dense thickets; it is a common tree on Indian coasts; it is perfectly capable of distribution by ocean-currents (fruits almost certainly belonging to this genus occur in various ocean-drifts); it is also probably capable of dispersal by means of frugivorous birds, as seeds of apparently species of *Premna* have been found in the crops of such.

On the other hand, *Gloriosa* is more likely to have been introduced; not only is it a showy plant, but natives of India believe that its roots possess powerful poisonous properties. It must be pointed out, however, that its seeds are capable of dispersal by winds, though it may be doubted whether they are capable of being carried so far as 130 miles, which is the distance of Anderut from the mainland. But it was not being cultivated where it was gathered, nor was it in cultivation elsewhere.

Pandanus odoratissimus may have been introduced because of its sweet scent. But it is very general in the archipelago; it is not cultivated by the islanders, and they do not make any use of it; species of *Pandanus* are amongst the most usual of ocean-distributed plants elsewhere; and it may be held as almost certain that it has been introduced by ocean-currents.

The grass that Mr. Hume took to be the common dubh-grass may have been introduced purposely, since some of the islanders have a few cattle; but it is far more likely, even if it was dubh, which it is extremely probable it was, to have been introduced as a weed.

While, then, all 16 may have been intentionally introduced by man, it is more probable that 7 (*Cæsalpinia*, *Morinda*, *Cynanchum*, *Tylophora*, *Ipomœa*, *Premna* and *Pandanus*) have arrived independently, and just possible that 5 others (*Calophyllum*, *Thespesia*, *Mucuna*, *Calotropis* and *Gloriosa*) may also have arrived independently. The remaining 4 (*Plumbago*, *Datura*, *Stachytarpheta* and *Cynodon*) have either been introduced intentionally or have been introduced as weeds, and so certainly owe their presence in the islands to the agency of man.

But, besides these four, there are 27 other species that are or may be weeds of cultivation, and as such are extremely liable to be dispersed by man involuntarily. For all in the following list this is without doubt the principal means of dispersion, and for the majority it is unquestionably the only possible means.

Some, however, for which other modes of distribution have been observed or claimed require discussion in detail. The list is as follows:—

Cleome viscosa.
Sida humilis.
Abutilon indicum.
Cardiospermum Halicacabum.
Crotalaria verrucosa.
Vernonia cinerea.
Ageratum conyzoides.
Wedelia calendulacea.
Wedelia biflora.
Crepis acaulis.
Physalis minima.
Barleria Prionitis.
Barleria cristata.
Rungia parviflora.
Peristrophe bicalyculata.
Leucas aspera.
Bœrhaavia repens.
Aerua lanata.
Achyranthes aspera.
Euphorbia pilulifera.
Phyllanthus maderaspatensis.
Acalypha indica.
Oplismenus compositus.
Setaria verticillata.
Andropogon contortus.
Apluda aristata.
Eleusine ægyptiaca.

The species of Compositæ at once suggest the possibility of wind distribution. But three of them, the *Ageratum* and both the *Wedelias*, are not provided with the feathery pappus favourable for wind-dispersal, and though Dr. Treub has made it abundantly evident that certain Compositæ may have their fruits wafted over 12—20 miles of intervening sea[1], it remains true, as M. A. DeCandolle has shown, that this is not very usual[2], and it seems doubtful whether the pappus of the *Vernonia* at all events is capable of supporting its fruit during a flight of 130 miles. The pappus of *Crepis* is, however, long and silky; and though its fruit is by no means so well adapted for aerial dispersion as the seeds of the Milk-worts are, it must be recollected that wind-dispersion is just possible for this species. Mr. Hemsley claims for *Boerhaavia* a dispersal by means of ocean-currents [3] and, considering the nature of their seeds and the facts of their distribution, the same dispersal may be claimed for *Aerua* and *Achyranthes*. But the evidence is not altogether conclusive. All three were collected by Mr. Hume on an island which is not inhabited; so far then the contention above stated is supported. But their fruits have not been recognized in ocean-drifts; and though this does not by any means preclude the possibility of their being present, no more does their existence on this uninhabited island prevent them from having been possibly introduced by man. Two are cosmopolitan weeds of the tropics; the third is widely spread in the tropics of the old world; all three are species whose seeds or fruits would naturally be found in the notoriously dirty grain sold in Indian bazars, and the Laccadive Islanders purchase their grain-supply for the season in India where these weeds abound. Then, though Betrapar is not inhabited, the people of other islands visit it to get its coco-nuts and to collect birds' eggs. The presence of shelter-huts on the island indicates that these visits are of some duration, and altogether the conditions necessary to explain the presence of these three weeds without having recourse to the hypothesis of ocean-dispersal are abundantly fulfilled.

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[1] Ann. Jard. Bot. Buitenzorg, vol. vii, p. 219.

[2] Géographie Botanique Raisonée, p. 702.

[3] Hemsley: "*Challenger*" Reports; Botany, Vol. i, part iii, page 44.

The grasses too suggest at once the possibility of wind-distribution. But none of those in the list are adapted (as an *Imperata* or a *Saccharum* would be) for this method of distribution. Some species of *Panicum* have been supposed to be distributed by the sea, but the *Oplismenus* and the *Setaria*, whose general resemblance to the *Panica* suggests for them this possibility, are most distinctly not coast plants on the mainland, and the hypothesis can hardly be applied to them. The *Apluda* and the *Andropogon* are, however, both coast and inland plants; for them therefore, though it is by no means probable, a sea-dispersal is just possible. Then any, or all, of them may conceivably have been distributed by marsh birds if their grains had happened to fall on the mud where these birds were wading. But it is far more probable that all the grasses mentioned here are, as *Eleusine ægyptiaca* certainly is, to be accounted for in the Laccadives as weeds of cultivation.

Man then has almost certainly either intentionally or unintentionally introduced 43 species, or 54 per cent., of Laccadive flora. At the same time it is possible that he may have introduced as many as 63 species, or 78¾ per cent. of the whole, while the probability is that man has introduced 56 species, or 70 per cent. of the whole flora. Conversely, the number of species that may have been introduced otherwise than by human agency is 37, or 46 per cent. of the flora; on the other hand, the number of species whose introduction has almost certainly been independent of man's agency is only 17, making 21¼ per cent. of the flora; the number of species whose appearance is probably independent of man is 24, or 30 per cent. of the flora.

Of the 17 species that have almost certainly not been introduced by man the following five are inland plants:—

Vitis carnosa.
Herpestis Monniera.
Oldenlandia diffusa.
Nephrodium molle.
Nephrolepis tuberosa.

The introduction of *Vitis carnosa* is almost certainly due to the agency of frugivorous birds, which have eaten its fruits on the mainland and deposited the undigested seeds on the island where it occurs. Still it has to be remembered that fruits belonging to this natural order have been found in ocean-drifts,[1] and that seeds of species whose fruits have been cast up by the sea have subsequently germinated.[2] Besides the wild vine, frugivorous birds, as has already been said, may have introduced *Premna;* but, whereas for the vine the probability of bird-introduction is greater, for *Premna* the probability is in favour of introduction by the sea.

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[1] Hemsley: "*Challenger*" Reports; Botany, vol. i, part iii, p. 290.

[2] Lefroy: Bot. Bermuda, p. 61.

Herpestis and *Oldenlandia* are marsh plants with small seeds that might easily be conveyed in pellets of mud attached to the feet of wading birds. They clearly may owe their introduction to this agency, and are the only Laccadive species for which this mode is the only conceivable one. But this agency may possibly account for the introduction of some of the grasses as well, and by no means improbably explains, at least in part, the distribution of one of the sedges.

The spores of both ferns are almost certainly wind-introduced; the wind probably also explains the presence of *Cynanchum* and *Tylophora*, not improbably the presence of *Crepis* and *Calotropis*, and possibly, though this is less likely, the presence of *Gloriosa*.

The other twelve are coast species, and have almost certainly been all introduced by the sea. They are—

Suriana maritima.
Guettarda speciosa.
Launea pinnatifida.
Scævola Koenigii.
Tournefortia argentea.
Ipomœa biloba.
Hernandia peltata.
Euphorbia Atoto.
Cyperus arenarius.
Cyperus pennatus.
Spinifex squarrosus.
Lepturus repens.

One of them (*Cyperus pennatus*) occurs on mud flats in tidal estuaries, as well as on open shores, and may owe its distribution at least in part to the feet of wading birds. For the others the distribution is clear. But there are several species concerning which the evidence is hardly less doubtful. These are—

Cæsalpinia Bonducella.
Morinda citrifolia.
Ipomœa grandiflora.
Premna integrifolia.
Pandanus odoratissimus.
Cocos nucifera.

But there are others still, all already alluded to and more or less exhaustively discussed, for which an ocean-distribution is quite conceivable. They are—

Calophyllum inophyllum.
Thespesia populnea.
Bœrhaavia repens.
Aerua lanata.
Achyranthes aspera.
Apluda aristata.

and there are a few others for which the possibility is so slight that they need not be again mentioned.

The number and proportion of the species that have been certainly, or possibly, or probably influenced in their introduction by the various agents of distribution are more easily appreciated when shown in the form of a table—

TABLE I.—Modes of introduction of Laccadive Plants.

INTRODUCED BY	CERTAINLY.		POSSIBLY.		PROBABLY.	
	No. of Sp.	Per cent.	No. of Sp.	Per cent.	No. of Sp.	Per cent.
Man	43	54 %	63	78¾ %	56	70 %
The Sea	11	13¾ %	22	27½ %	17	21¼ %
Birds	2	2½ %	5	6¼ %	3	3¾ %
Winds	2	2½ %	7	8¾ %	4	5 %

The general distribution of the flora is, next to its means of introduction, the feature of greatest interest. The majority of its constituent species, cultivated and wild alike, are cosmopolitan in the tropics, while there is not a single species that the flora does not share with the adjacent Indian mainland, and only five that it does not share with Ceylon. Even *Lepturus repens*, for which the archipelago is a new, but not an unexpected locality and which has not been hitherto included in Indian lists, was collected by Mr. Hume at Vingorla Rocks near the Goanese coast during the same voyage as that in which he obtained his Laccadive collection.[1]

It ought to be observed that the date of Dr. Alcock's visit—the first week of March—was only a fortnight later in the season than that of Mr. Hume's visit, yet in Kiltan Dr. Alcock found a wild vine growing in great profusion which Mr. Hume, who visited the same island, did not observe. The list contains other instances, but this is the most striking, and it at once suggests the possibility of this species having been introduced during the interval between 1875 and 1889, and indicates one of the possible interests that may underlie a comparison of this with subsequent lists.

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[1] This is the species referred to in Mr. Hume's paper in "Stray Feathers" as perhaps a species of *Hemarthria*. Dr. King, who has very kindly examined the specimens along with me, assents to the identification now made. Dr. Trimen records it from Ceylon (Cat. of Ceylon Plants, p. 110, [1885]); it was not known from Ceylon at the time that Dr. Thwaites' "Enumeration of Ceylon Plants" was published.

The subjoined table exhibits the distribution of the various species that compose the flora (species that are purely cultivated are marked†)—

TABLE II.—*Distribution of Laccadive Plants.*

NAME.	India.	Ceylon.	Nicobars.	Andamans.	Burma.	Malacca.	Malay Archipelago.	Australia.	Polynesia.	America.	Africa.	Mauritius.	Keeling Islands.	Chagos Islands.
Cleome viscosa	×	×	×	×	×	×	×	×	...	×	×	×	...	...
Calophyllum inophyllum	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Sida humilis	×	×	...	×	×	×	×	...	...	×	×	×	...	...
Abutilon indicum	×	×	...	...	×	...	×	×	...	×	×	×	...	...
Thespesia populnea	×	×	×	×	×	×	×	×	×	×	×	×	..	...
†Citrus acida	×	×	...	×	×	×	×	×	×	×	×	×	...	...
Suriana maritima	×	...	×	..	...	×	×	×	×	×	×	×	...	×
Vitis carnosa	×	×	×	...	×	×	...	...	...	...	...	...	...	...
Cardiospermum Halicacabum	×	×	×	×	×	×	×	×	×	×	×	×	...	...
†Moringa pterygosperma	×	×	...	×	×	×	×	...	...	...	...	×	...	...
Crotalaria verrucosa	×	×	...	...	×	×	×	×	...	×	×	×	...	...
Clitoria Ternatea	×	×	×	×	×	×	×	...	×	×	×	×	...	...
Mucuna capitata	×	...	...	...	...	...	×	...	...	...	...	..	...	...
Cæsalpinia Bonducella	×	×	×	×	×	×	×	×	×	×	×	×	×	...
†Tamarindus indica	×	×	×	×	×	×	×	×	×	×	×	×	...	...
†Punic granatum	×	...	...	...	×	×	×	×	×	×	×	×	...	...
†Carica Papaya	×	×	×.	×	×	×	×	×	×	×	×	×	...	...
Oldenlandia diffusa	×	×	×	×	×	×	×	...	...	...	...	...	...	...
Ixora Bandhuca	×	×	...	..	×	...	...	...	...	...	...	...	...	...
Morinda bracteata	×	×	×	×	×	×	×	...	...	...	...	...	...	...
Guettarda speciosa	×	×	×	×	×	×	×	×	×	...	×	×	×	×
Vernonia cinerea	×	×	×	×	×	×	×	×	...	...	×	×	...	×
Ageratum conyzoides	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Wedelia calendulacea	×	×	×	×	×	×	×	...	...	...	...	...	...	...
Wedelia biflora	×	×	×	×	×	×	×	×	×	...	×	...	...	...
Crepis acaulis	×	...	...	...	×	...	...	...	...	...	...	...	...	...
Launea pinnatifida	×	×	...	...		...	...	...	...	...	×	×	...	...
Scævola Koenigii	×	×	×	×	×	×	×	×	×	...	..	×	...	...
Plumbago zeylanica	×	×	...	..	×	×	×	×	×	...	×	×	...	.
Calotropis gigantea	×	×	...	...	×	×	×	...	...	...		...	...	...
Cynanchum alatum	×	...	...	..	...	...	...	..	...	...	...	...	...	...
Tylophora asthmatica	×	×	...	.	×	×	×	...	...	...	...	×	...	...
Tournefortia argentea	×	×	×	×	×	×	×	×	×	...	×	×	×	...
Ipomœa grandiflora	×	×	...	...	...	...	×	×	×	...	×	×	...	...
†Ipomœa Batatas	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Ipomœa biloba	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Physalis minima	×	×	×	×	×	×	×	×	...	×	×	...	...	...
†Physalis peruviana	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Datura fastuosa	×	×	×	×	×	×	×	...	...	×	×	×	...	...
Herpestis Monniera	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Barleria Prionitis	×	×	...	...	×	×	×	...	...	...	×	×	...	...
Barleria cristata	×	×	...	...	×	×	×	...	..	...	...	×	...	...
Rungia pectinata	×	×	...	×	×	×	×	...	...		...	...	...	...
Peristrope bicalyculata	×	...	×	...	×	...	...	...	...	...	×	...	...	...

TABLE II.—Distribution of Laccadive Plants—continued.

NAME.	India.	Ceylon.	Nicobars.	Andamans.	Burma.	Malacca.	Malay Archipelago.	Australia.	Polynesia.	America.	Africa.	Mauritius.	Keeling Islands.	Chagos Islands.
Stachytarpheta indica	×	×	×	×	×	×	×	...	..	×	.	.		×
Premna integrifolia	×	×	×	×	×	×	×	×	×	...	..	×	...	...
Leucas aspera	×	...	...	...	×	...	×	...	...	...	...	×	...	...
Boerhaavia repens	×	×	×	×	×	×	×	×	×	...	×	×	×	...
Aerua lanata	×	×	×	×	×	×	×	...	...	...	×	×	...	...
Achyranthes aspera	×	×	×	×	×	×	×	×	×	×	×	×	×	...
Hernandia peltata	×	×	×	×	×	×	×	×	×	...	×	×	...	...
Euphorbia Atoto	×	×	×	×	×	×	×	×	×	...	...	...	...	...
Euphorbia pilulifera	×	×	×	×	×	×	×	×	×	×	×	×	...	×
†Phyllanthus Emblica	×	×	×	×	×	×	×	...	...	...	...	...	...	...
Phyllanthus maderaspatensis	×	×	...	...	...	...	...	×	...	...	×	×	...	×
Acalypha indica	×	×	×	×	×	×	×	...	...	...	×	×	...	...
†Ricinus communis	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Ficus bengalensis	×	×	...	...	×	×	×	...	...	...	...	...	...	...
†Artocarpus incisa	×	×	...	...	...	×	×	×	×	×	×	...	...	...
†Musa sapientum	×	×	×	×	×	×	×	×	×	×	×	×	...	...
†Agave vivipara (?)	×	×	..	...	×	×	×	×	×	×	×	×	...	...
†Dioscorea sativa	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Gloriosa superba	×	×	×	×	×	..	...	...	...	...	...	...	...	...
†Areca catechu	×	×	×	×	×	×	×	×	×	×	×	...	...	...
†Cocos nucifera	×	×	×	×	×	×	×	×	×	×	×	...	×	...
Pandanus odoratissimus	×	×	×	×	×	×	×	×	×	×	×	×	...	...
†Colocasia antiquorum	×	×	×	×	×	×	×	×	×	...	×	×	...	...
Cyperus arenarius	×	×	...	...	×	...	...	...	...	...	...	...	...	...
Cyperus pennatus	×	×	×	×	×	×	×	×	×	...	×	×	...	...
Oplismenus compositus	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Setaria verticillata	×	×	×	×	×	×	×	×	×	×	×	...	...	...
Spinifex squarrosus	×	×	×	×	×	×	×	...	...	...	...	...	...	...
Andropogon contortus	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Apluda aristata	×	×	...	...	×	×	×	...	...	...	...	...	...	...
Cynodon dactylon (?)	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Eleusine ægyptiaca	×	×	×	×	×	×	×	×	×	×	×	...	...	...
†Eleusine Coracana (?)	×	×	...	...	×	..	...	...	...	...	×	..	...	...
Lepturus repens	×	×	..	...	...	...	...	×	×	...		×	×	...
Nephrodium molle	×	×	×	×	×	×	×	×	×	×	×	×	...	...
Nephrolepis tuberosa	×	×	×	×	×	×	×	×	×	×	×	×	...	...

The cultivated species are not taken into account in the calculations given below. Even when they are excluded, we find that 18 species extend to Africa, America, Australia, and Polynesia, as well as to Asia; four species extend to all the great geographical divisions except Polynesia; one to all the divisions except Australia. Three that occur in Asia, Africa, and America are excluded both from Australia and from Polynesia.

On the other hand, 11 that are common to all the other divisions are absent from the New World, and no fewer than seven of these belong to the coast flora which is characterized by ocean-distribution.[1] Two others (*Vernonia* and the herbaceous *Phyllanthus*) are excluded from Polynesia also, but both of these are simple weeds of cultivation. One coast species that extends both to Australia and Polynesia (*Euphorbia Atoto*) does not reach America, and at the same time appears to be absent from the African coast. Five species extend from Asia to Africa only. One of them (*Peristrophe bicalyculata*) is a very common Indian weed that extends into Assam and into Burma, but, though it appears in the Laccadive islands, it has not hitherto been reported from Ceylon. Another (*Launea pinnatifida*) is a coast species much less extensively distributed than species of this class usually are; it appears to be confined to the coasts of India and Ceylon, to Mauritius, and to the eastern coast of Africa.[2] Three species that are characteristic of Continental Asia appear in Mauritius. These are *Tylophora asthmatica*, *Barleria cristata*, and *Leucas aspera;* their distribution as weeds probably accounts for the presence there of all three.[3] The other 15 are confined to Asia.[4]

The following table shows more compactly the constitution of the Laccadive flora:—

TABLE III.—*Constituents of the Laccadive Flora.*

Distributional Features.	No. of species.	Per cent.
Cultivated species	17	21¼ %
Cosmopolitan in the Tropics	8	22½ %
Almost Cosmopolitan : not in Polynesia	4	5 %
Ditto ditto : not in Australia	1	1¼ %
Nearly ditto : not in Australia nor in Polynesia .	3	3¾ %
Tropics of old world and Polynesia	11	13¾ %
Tropics of old world alone	2	2½ %
Asia, Australia and Polynesia only	1	1¼ %
Continental Asia and Continental Africa	5	6¼ %
Ditto and Mauritius	3	3¾ %
Confined to Asia	15	18¾ %
	80	100 %

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[1] These sea-coast species not extended to the new world are *Guettarda speciosa, Scævola Koenigii, Tournefortia argentea, Hernandia peltata, Premna integrifolia, Cyperus pennatus* and *Lepturus repens.* But to these have to be added *Morinda bracteata, Launea pinnatifida, Euphorbia Atoto, Cyperus arenarius.* and *Spinifex squarrosus*, which are circumscribed in their distribution even within the eastern hemisphere. And, besides these, *Thespesia populnea*, which is so common on tropical shores in the old world, is only known in the West Indies as an introduced and naturalized species. Altogether therefore 13 of the Laccadive sea-coast species are plants confined to the old world.

[2] *Eleusine Coracana,* one of the cultivated species, has also this limited Asiatic and African distribution.

[3] *Moringa pterygosperma,* a cultivated species, is also limited to Asia and Mauritius.

[4] *Phyllanthus Emblica,* which is cultivated, is also confined to Asia.

The distribution of the 15 species confined to Asia varies somewhat in different instances. This is more easily understood when the facts are tabulated—

TABLE IV.—Laccadive plants confined to Asia.

Extended to the Laccadives from	No. of species.	Per cent.
India, Burma, Malaya, Ceylon	9	11¼
India, Burma, Ceylon	3	3¾
India, Burma	1	1¼
India, Malaya	1	1¼
India alone	1	1¼
TOTAL	15	18¾

The class of plants to appear first in the archipelago were those belonging to the coast flora, since they could arrive almost as early as wind-conveyed species, and would find the conditions offered by newly emerged islets favourable for their existence long before these became favourable for wind-dispersed forms, while birds, and, as a consequence, bird-conveyed species, would be unlikely to alight on the islands before the establishment on them of a sea-conveyed flora inviting them to do so. A reference to TABLE I shows how completely this theoretical conclusion is borne out by the present character of the flora; independently of those species whose appearance has been due to human agency, the coast species—which the physical conditions are favourable for—are, as compared with the inland species, more numerous almost in the proportion of 3 : 1; as compared with wind-introduced species alone—the introduction of whose seeds or spores may have begun equally early, but for whose existence the conditions are less favourable even now—more numerous in a proportion of over 4 : 1; at the same time bird-conveyed species for which the conditions are not more unfavourable than they are for wind-conveyed species, but which have probably been later of introduction—are less numerous than wind-conveyed species.

While there is this more than general agreement with the results that a consideration of the probable physical history of the islands would lead us to expect, the numbers discussed are too small to render the statistics conclusive. But the further evidence necessary is to be obtained by a comparison of the list of plants already given with a list of the seeds and fruits picked up and a list of the plants observed growing on an uninhabited island just beginning to receive a flora such as Krakatau when visited in 1886 by Dr. Treub of Buitenzorg.[1] All the vegetation on this island was destroyed by the great eruption of 1883, and the factor of human interference in its refloration is eliminated, as it is quite unvisited by man and is quite uninhabitable.

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[1] KRAKATAU is a volcanic island in the Straits of Sunda, the craters of which were unusually active from May till October 1883, and which was in August (26th, 27th and 28th) of that year the scene of an eruption of extraordinary violence that greatly altered the physiographical features of the island and completely destroyed the whole of its previously luxuriant vegetation. Dr. Treub visited the island in 1886 to ascertain what species had during the interval reached the island and what light these might throw on the processes of afforation. In this case the agency of man is completely eliminated, and only those of the sea-birds, or winds are at work. Dr. Treub's paper has been already referred to; it is to be found in Ann. Jard. Bot. Buitenzorg, vii, 213.

On the shore Dr. Treub found seeds or fruits of—

Heritiera littoralis.
Terminalia Catappa.
Cocos nucifera.
Pandanus (2 species).
Barringtonia speciosa.
Calophyllum inophyllum.

Growing there he found young plants of—

Erythrina (species).
Calophyllum inophyllum.
Cerbera Odallam.
Scævola Kœnigii.
Ipomœa biloba.
Hernandia peltata.
Cyperaceæ (2 species).
Gymnothrix elegans.

The last-named species is clearly a wind-conveyed grass; the others are as clearly all sea-conveyed species. On the mountain proper he found *Scævola Kœnigii* again, also *Tournefortia argentea*, four palpably wind-conveyed *Compositæ*, with two grasses, eleven ferns, and two mosses,—all evidently (except *Scævola* and *Tournefortia*) wind-dispersed. The general similarity between the coast list and the coast section of the Laccadive list is very striking and becomes all the more so when we consider that Krakatau is only 21 miles from Java, and 10 and 20 miles from Siebesie and Sumatra respectively, and has land on every quarter, while Anderut, the nearest island of the Laccadive group to the mainland, is 130 miles from the Indian coast, and the archipelago, as a whole, has land on one side only.

While the species that constitute the coast flora are the first to arrive on newly emerged coral islands, they form the section of the flora least liable to subsequent alteration. Thus, while Dr. Alcock has brought to our notice 16 species that were not seen by Mr. Hume, none of these belong to the coast flora, while three — the ferns and *Cynanchum* — are almost certainly wind-conveyed species, and three — *Vitis*, *Oldenlandia* and *Herpestis* — are almost certainly bird-introduced. The others are all instances of plants intentionally or unintentionally introduced by man.

The extremely low percentage of vascular cryptogams in the Laccadive list, as compared with the percentage found by Dr. Treub on Krakatau[1], deserves remark. There the vascular cryptogams formed 46½ per cent. of the whole and 62 per cent. of the interior flora to which they properly belong. In the Laccadives, on the contrary, they form but 2½ per cent. of the whole, or 3 per cent. of the inland flora.

The state of affairs on Krakatau is, as Dr. Treub shows, in accordance with expectation and in agreement with the evidence afforded by mountainous volcanic islands elsewhere. These become first stocked with a wind-conveyed cryptogamic flora which survives the more readily that its constituents are not highly specialized, and which prepares the way for a more highly specialized phanerogamic flora. But the larger proportion of the phanerogams that do reach such islands are ocean-distributed and littoral species that cannot ascend beyond a certain height, those whose fruits or seeds are adapted for very distant wind-conveyance being comparatively few in number, and the proportion of such fruits or seeds that escape from perishing in the ocean *en route* being very small. Hence, in such islands the proportion of cryptogams to phanerogams always remains high, and is increased in a direct ratio with the distance of the island from adjacent land, and in an inverse ratio with the suitability of the surface of the island for the support of vegetable life.

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[1] Ann. Jard. Bot. Buitenzorg, vii, 218.

The conditions that prevail in newly emerged coral islets are very different. Their low elevation in itself diminishes the chances of wind-conveyed spores alighting on them, and is in itself unfavourable for the germination of many of the spores that do alight. Then, for those that have succeeded in alighting and that have germinated the struggle for existence is intensified by the simultaneous arrival of another class of species, independently conveyed, more highly specialized, with its individual species more largely represented, and for which the same physical conditions are in the highest degree favourable. These conditions appear sufficiently to explain the difference of the proportions in Krakatau and in the Laccadives.

In concluding, the writer wishes to acknowledge his great indebtedness to Dr. Alcock for much information concerning the present flora of the Laccadives and to Dr. King for numerous valuable suggestions while analysing its constituents.

On the Life History of a Himalayan Gymnosporangium

(*G. CUNNINGHAMIANUM, NOV. SP.*)

BY

SURGEON-MAJOR A. BARCLAY, M. B.

BENGAL MEDICAL SERVICE.

I had long suspected that the Aecidium on the wild pear (*PyrusPashia* , Ham.), which is in certain years very common in the neighbourhood of Simla (Western Himalayas), was related to a Gymnosporangium on *Cupressus torulosa,* Don., and I noted the probable connection in a paper submitted to the Asiatic Society of Bengal in 1887,[1] and incidentally in a paper on *Caeoma Smilacis* in these Memoirs, Part IV; but it was not until the spring of 1889 that I succeeded in proving this by experiment.

The wild pear is abundant in Simla, but the cypress is not. The Aecidium is consequently by no means common, except in the neighbourhood of affected cypress trees. An occasional aecidial patch may, however, be found at a considerable distance from any cypress tree.

In the following paper I propose first to describe the characters of the fungus as it occurs on its two hosts, and then to pass on to a consideration of its title to be considered a distinct species.

I. Teleutosporic stage on Cupressus.

The teleutospore beds are hemispherical dark brown compact bodies during dry weather, and are found on the ultimate small branches, as well as on older twigs of 4 to 5 mm. in diameter. Their appearance during dry weather is represented in Fig. 1, Plate I. During very moist weather these beds swell up enormously into gelatinous masses, and soon assume a yellow ochre colour, due to a plenteous formation of sporidia. The swelled condition of the teleutospore beds is shown on Fig. 2, Plate II. During heavy rain the gelatinous masses fall off from the tree. Teleutospore beds may be found during the greater part of the year. I have missed them only during August to November.

The *mycelium* producing these spores is perennial, and causes some slight, but by no means conspicuous, thickening of the parts involved. It ramifies between the cells of the bark tissue, and penetrates down to the central woody tissue, but does not apparently enter the latter. Near spore beds the mycelium is extremely abundant, but, as we recede from this area the amount of mycelium quickly diminishes. Where abundant near beds of teleutospores the hyphae contain orange-red oil globules; but elsewhere the mycelium is colourless. It never appears to enter the cells, and there are consequently no haustoria. The stimulant effect of the mycelium on adjoining cells is shown in their increased size. The bark cells, just below the outer sub-epidermal palisade-like cells, as seen in transverse sections of the youngest twigs, are normally about 30μ in diameter, whilst in areas pervaded by mycelium these present a diameter of about 50μ.

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[1] ournal of the Asiatic Society of Bengal, Vol. LVI, Part II, No. 3, 1887.

The teleutospores are slender spindle-shaped yellow bodies, on long stalks covered with a substance capable of swelling exceedingly when moistened (Fig. 5, Plate II). Sometimes, when the spore beds are moist, and some spores are scraped off, the central axile stalk comes out of the gelatinous sheath as a very slender stalk. In such cases there is a characteristic disc between the base of the spore and the stalk (Fig. 9, Plate II). There is no appreciable constriction at the septum. Each cell of the spore exhibits a central nucleolar space, or nucleus, and near the septum each cell has two germ spores. The fresh spores, when scraped off their beds and examined immediately in water, measure $75{\cdot}6 \times 25{\cdot}2\mu$. They have a thin membrane, not usually thickened anywhere, and without any external markings. Sometimes a slight thickening of the apex, with a short blunt conical process, may be seen.

When placed in water the spores germinate very rapidly. In one cultivation spores were placed in water at 9 A.M., and at 2 P.M. many had thrown out promycelia 60 to 80μ in length (April). I have looked carefully for the second kind of spore, with short stalks, thin walls, lighter coloured, and much constricted at the septum, as described by Dietel[1] in most species investigated by him, but have not found them. I could not find any second form of spore decidedly different from the form I have described. At first each cell in germinating throws out two commencing promycelia from the pores near the septum, the four forming a cross. Only one promycelium from each cell, however, continues to develope, the other remaining undeveloped (at least in water cultivations). All the coloured contents of each cell of the spore wanders in mass into the very end of the developing promycelium, and keeps to the end, however long the tube may grow, leaving the proximal end of the tube quite colourless. Sometimes in a water cultivation the tubes attain a very considerable length; but they are usually short (Fig. 3, Plate II). The promycelial tube measures from 8 to 11μ in diameter: it is usually widest at the end, where the coloured contents have accumulated. The end now invariably, so far as my observations extend, divides into four compartments by four septa, the lowest just including the coloured contents (Figs. 3, 4, Plate II). Stout sterigmata are formed from each cell, and very frequently all these are on one side of the promycelium, *i.e.* in a row one above another (Fig. 3, Plate II). This is not invariable, but usual. Each sterigma bears a sporidium (Fig. 4, Plate II), and therefore four such are formed by each promycelium. The sporidia are orange-red and oval, and measure from $15 \times 9\ \mu$ to $22 \times 14\ \mu$. When all the four sporidia have been formed, the promycelium is almost colourless.

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[1] Ueber das Vorkommen von Zweierlei Teleutosporen bei der Gattung Gymnosporangium, Hedwigea, 1889, Heft 2, p. 99.

When many spores are germinating in a very small quantity of water, only those which have germinated earliest proceed to the normal development of sporidia: those which germinate later (48 hours) usually exhibit the abnormal process first described by Kienitz-Gerloff, and which I have elsewhere[1] described as characteristic of *Caeoma Smilacis,* and a Uromyces on *Solidago Virgaurea;* that is to say, after the end of the promycelial tube has divided into four cells, the cells round off and become detached (Fig. 8, Plate II). This abnormal growth is not confined to spores of any peculiar form, for, as I have already noted, there is only one kind of spore. I have not seen such detached cells germinate.

In connection with the germination of these spores I may draw attention to another curious and interesting variation. In water cultivations I have occasionally seen the condition represented in Fig. 10, Plate II, in which a terminal cell of the promycelium has developed a very long slender tube, in every respect resembling the germ tube of a sporidium. Such growth obviously suggests the suppression of sporidial formation.

The sporidium germinates very quickly, after ripening (Fig. 6 c, Plate II). The formation of secondary sporidia in water cultivations is not uncommon.

II. Æcidial stage on *Pyrus Pashia,* Ham.

This stage of the life history of the fungus I have already described in the Journal of the Asiatic Society of Bengal,[2] but, in order to complete the present paper I will describe it again. It is remarkable that this member only of the several species of allied fruit-trees occurring in this region harbours the parasite. I have frequently looked in vain for it on other species. Another noteworthy feature is that, whilst in some years the Æcidium occurs abundantly, in others it is rarely found. As the teleutospores are formed abundantly every year, this can only be accounted for on the assumption that certain well-defined atmospheric conditions must concur to enable the sporidia to attack the leaves. In 1885 the Æcidium was very common; in 1886 it was remarkably rare; in 1887 it was again fairly abundant, though not nearly so common as in 1885; and now, in 1889, it is again common (I was absent from India during 1888). Mr. W. L. Dallas, Assistant Meteorological Reporter to the Government of India has very kindly furnished me with meteorological data for the months April to August during these years. In 1885 the rainfall was in every month, except July, above the average, and relative humidity was likewise above the average in every month except June. In 1886, a year in which the æcidia were rare, the rainfall was deficient in all months except July, and relative humidity was deficient in April and June, and excessive in May, July, and August. In 1887, rain was below the average in all months, and relative humidity in April to June, and excessive in July to August. In 1889 rainfall was deficient in April and May, but excessive in June and July. But perhaps the number of days on which rain fell during the months April to June, when attack usually occurs, has a more powerful influence in this respect. In 1885, during these three months rain fell on 40 days, in 1886 on 27 days, in 1887 on 26 days, and in 1889 on 31 days. The æcidia were much more abundant in 1885 and 1889 than in 1886 and 1887.

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[1] These Memoirs, Part IV.

[2] Vol. LVI, Part II, No. 3, 1887.

The fully developed æcidia appear usually during July to September, *i.e.* during very wet weather; but the spermogonial stages are often met with earlier, even as early as April, the rains not setting in until the end of June. A considerable interval of about a month, or even more, intervenes between the eruption of spermogonia and the appearance of the æcidia.

So far as my observations extend, the parasite occurs only on the leaf blades and never on the petioles or young fruit. The first foliage of the year is put forth very early in spring, whilst a subsidiary foliage occurs during the rains; but generally only the older leaves of the first foliage are attacked. This, however, is probably due to the exhaustion of the supply of teleutospores, as in my artificial inoculations I have always found the young paler leaves very susceptible to attack. The two sets of leaves are easily distinguished during the time the æcidia are present, as the older leaves are much darker green in colour. Well-defined patches, orange-red above and yellowish below, are formed on attacked leaves (Figs. 1,2, Plate II). At first spermogonia only are formed, all invariably apparently on the upper surface of the leaf blade, and at this time he at tacked parts are slightly thickened, the depth of the tissues being about 0.41 m.m., whilst normally it is about 0.17 m.m. to 0.19 m.m. After a considerable interval æcidia are produced on the lower surface of the leaf blade, and then the attacked areas are considerably swollen, the depth of the tissues being about 0.90 m.m. This hypertrophy is due to a peculiar proliferation and transformation of the spongy tissue cells, and not to any variation in the palisade cells, of which there are two layers. The mycelium, which ramifies mainly throughout the spongy tissue cells, causes them to enlarge very considerably, and especially at right angles to the surface of the leaf, forming long oblong cells, as seen in transverse section of the leaf, closely applied latterly (Fig.3, Plate III). These long cells are from 65 to 107μ in length, by 20 to 30μ in beadth, while the true palisade cells are about 44μ in length. Some of these long cells stain deeply with all ordinary stains, as will be seen in the figure, whilst others do not take the stain so readily.

The *æcidia* are long, somewhat flask-shaped or cylindrical bodies, very deeply sunk in the hypertrophied leaf tissue. Their bases, indeed, rest almost directly upon the palisade cells (Fig. 3, Pl. II). Each æcidium is situated on a minute papilla. The portion of the structure sunk beneath the level of the lower epidermis is about 0·756 m.m. in length, whilst the part projecting free is from one to 2 m.m. in length.

The *peridium* consists of a single layer of gray or pale dirty yellow cells, all, except those at the very summit of the free part, being considerably longer than they are broad. Those about the middle measure about 70×22μ (Fig. 11a, Plate II). These cells are beset, specially on the inner surface, with prominent short irregular ridges. They are united to one another in longitudinal series as follows. The upper end of each cell is bevelled like a chisel, the sharp edge overlapping internally the lower end of the cell above. The lower edge of each cell is similarly bevelled, but in the opposite direction, so that it overlaps the upper bevelled end of the cell below externally (Fig. 11 b, Plate). In teasing the tissue of the peridium it is seen that the cells are easily detached from one another laterally, but are firmly united to one another by their ends. The peridium dehisces by the formation of numerous irregular slits in its side, and also opens freely at the summit, the tube retaining its original form throughout the time the spores are being shed. A few æcidiospores may always be seen sticking in the meshes of these slits. The majority of the spores would appear to fall out through the opening in the summit.

The *æcidiospores* are round, or oftener oval, when free and moist, but when dry are often facetted. They are given off serially from a hymenium of long regular basidia; the hymenium measuring about 60μ in depth. They are pale brown, beset densely externally with minute tubercles, and measure when just wetted 28.6×24.6μ on an average (28μ diameter in round to 31× 25μ). Placed in water, they germinate readily, throwing out in 24 hours a long, much branched, germ tube (Fig. 12, Plate II). In such cultivations I could not assure myself that there were any slit-like openings in the endospore, as described by Rees,[1] nor any bulging inwards of the endospore opposite the germ pores, of which there are usually six, but sometimes eight.

The *spermogonia* are, as usual with this group of Uredines, very large and prominent. When ripe, their orifices are covered with a clear glistening sticky fluid, probably secreted by the well-developed tuft of paraphyses, which, arising from the very base of the structure, protrude about 50 to 60μ beyond the orifice. They are deeply sunk in the leaf tissue, their bases pushing down, and partly disintegrating, the upper palisade layer. The effect of Spiller's purple upon these structures is somewhat peculiar, as, while the sterigmata are coloured blue, the spermatia assume a brick-red colour. The spermatia are large and oval, measuring $8 \times 4\mu$. On several occasions I placed these spermatia in weak solutions of sugar, but never observed any yeast-like germination.

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[1] Die Rostpilzformen der deutschen Coniferen, Dr. Max Reess, Halle, 1869.

This completes the description of the fungus, and I now proceed to describe briefly the experiments I made to establish the connection between the two phases of existence of the parasite.

During March and April I frequently applied the sporidia obtained from the teleutospores on Cupressus to the leaves of Pyrus, and in most cases saw spermogonia developed in ten to fourteen days. The exact method of procedure was as follows. Small twigs of Pyrus were cut off under water, and then placed for a day or two under a glass shade in my laboratory, means being taken to keep the air within moist. In the meanwhile some dry teleutospore beds were scraped off from Cupressus, and put into a watch-glass containing water, the whole being kept in a moist atmosphere. In a very short time these beds swelled up extraordinarily into light tremulous gelatinous masses, and in 24 hours were covered with light yellow sporidia. These sporidia were then transferred with a sterilized glass rod to the leaves on the twigs of Pyrus under the glass shade, and left there until the earliest sign of attack was apparent. They were then put into an ordinary gardener's forcing-box. If kept too long under a glass shade, the leaves soon withered. Even in the forcing-box the leaves did not remain alive long enough for the production of æcidia, although numerous characteristic spermogonial patches were produced.

As I found it impossible to keep such twigs long enough alive to allow of the formation of æcidia, I tied some twigs of Cypress, on which there were teleutospore beds, on to three bushes of Pyrus in my garden, after the wet weather had set in. On each bush, after about a fortnight, many of the newly unfolded leaves exhibited bright orange-red spermogonial patches, and these went on to the formation of æcidia about a month later. The photograph, reproduced in Fig. 1, Plate 3, represents a twig taken from one of these bushes in my garden.

These experiments show quite conclusively that the *Pyrus æcidium* is related to the teleutospores which are borne on *Cupressus torulosa.*

With regard to the geographical distribution of this Gymnosporangium, I am sorry to say I have but little information. During an excursion into the interior of the Himalayas I found the Æcidium many miles from Simla. My friend Dr. D. D. Cunningham informs me that he found what appears to be the same æcidium at Almora in the Kumaun Hills in 1874, and he has kindly placed the drawings he made of it at my disposal (Figs. 13, Plate II, and 2, Plate III). I am told, not however with certainty, that *Cupressus torulosa* occurs there. After so long a time Dr. Cunningham could not himself remember whether any of these trees existed there. The measurements of his æcidiospores are 25·4 × 28·6, on an average. The whole length of his peridial tube is about 2 m.m., of which the submerged portion measured 0·5 m.m., leaving 1·5 for the projecting free gray part. In diameter the peridium measured 0·25 m.m. He represents in a figure, which I do not reproduce, a few spermogonia as occurring on the lower or æcidial surface. I have never myself seen spermogonia on the lower surface. The spermatia measured 3·17 × 6·35μ. These figures are sufficiently near mine to render it probable that the fungus is identical. It is quite possible, however, that the Almora species is distinct, more especially as there is some doubt whether *Cupressus torulosa* exists there.

It now remains for me to consider whether this Himalayan Gymnosporangium is identical with any of the hitherto described species. In the paper above referred to in the Journal of the Asiatic Society of Bengal, I tentatively named the species G. *clavariæforme*, mainly because the dehiscence of the peridium corresponded fairly closely with that of the latter species; but the discovery of the complete life-history of the fungus enables me to consider this point more fully, and I am now convinced that the Simla species is not identical with *G. clavariæforme.* Whilst the æcidium of *G. clavariæforme* occurs on several species, here it occurs invariably on one only. In the former the teleutospore beds are light yellow, even when dry, and when swelled are more cartilaginous than gelatinous: in the Himalayan species the dry beds are dark brown, and when moistened are essentially gelatinous. Moreover, the shapes of the swelled beds in the two varieties are very different. The teleutospores in both agree in shape, but whilst some of those of *G. clavariæforme* are light brown, all are pale yellow in Simla: the length of the spores in the former varies rom 65 to 95μ and the breadth from 14 to 16μ.: in the latter the length is 63 to 78, and the breadth 20 to 25μ. Again, whilst the former are said to have four pores to each cell, the latter have only two. The fine slit-like pits distributed over the endospore in *G. clavariæforme* are entirely absent in the Simla species. The promycelium in the former divides into three, forming three kidney-shaped sporidia, 10μ long: in the latter it divides very regularly into four, bearing four oval sporidia, 15 to 20μ long by 9 to 14μ broad. The æcidia in Simla are seen only on the leaves. The peridial cells about the middle in *G. clavariæforme* are 21 × 30 against 70 × 22μ in the Simla species. The æcidiospores in the former are 26 × 30μ, and have six germ pores, whilst in the latter the spores measure 28·6 × 24·6μ, but present, as a rule, the same number of pores. These differences make it clear, I think, that the Simla fungus is specifically distinct from *G. clavariæforme.*

Compared with *G. fuscum* (D. C.), Oerst., there are many differences especially in the dehiscence of the peridium.

But there are some points of similarity with *G. conicum* (Hedw. f.) (D. C.), Oerst., especially in the form of the teleutospore beds; but there are important differences. This species has two forms of teleutospore, with four pores to each cell; it has longer æcidia, opening in a different manner, and the æcidiospores are somewhat smaller than in the Himalayan species. The Himalayan species is therefore distinct from all these.

I have also carefully compared the Himalayan species with Farlow's description of the American species,[1] and feel persuaded that it is distinct from any one of them. There can be no doubt whatever that it is quite distinct from *G. Ellisii* (Berk.) and *G. biseptatum,* Ellis, both of which are characterized by the teleutospores being usually divided into more than two cells, and both of which, curiously enough, are the only other Gymnosporangia borne on species of Cupressus (*C. thyoides*).

A new name is therefore wanted for the Himalayan species, and I propose calling it *Gymnosporangium Cunninghamianum,* after my accomplished friend Dr. D. D. Cunningham, F.R.S.

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[1] The Gymnosporangia, or Cedar Apples, of the United States: W. G. Farlow, 1880.

Description of the Plates.

[Except where otherwise noted all × 350.]

PLATE I.—Photographs, natural size.

1. *Cupressus torulosa* with teleutospore beds as seen in dry weather.
2. *Ditto* *ditto* ditto as seen in rainy weather.

PLATE II.—1. Under surface of leaf with æcidia, natural size.

2. Upper surface ditto.
3. Germinating teleutospore : two promycelia.
4. Promycelium showing one sporidium.
5. Teleutospore shortly after immersion in water.
6. (*a*) Outline of terminal end of promycelium.
   (*b*) Ripe sporidium.
   (*c*) Germinating sporidium.
7. Germinating sporidium : three days in water.
8. Abnormal division of promycelium into cells, instead of forming sporidia : 48 hours in water.
9. Outline of teleutospore showing disc at base, × 900.
10. Abnormal germination of ends of terminal cells of promycelia into sporidial germ tubes.
11. Peridial cells, (*a*) as seen flat, (*b*) as seen in optic section, showing mode of union to one another.
12. Germinating æcidiospore.
13. Peridial cells and æcidiospores of æcidium from Almora (drawn by Dr. D. D. Cunningham).

PLATE III.—1. Photograph, natural size, of a shoot attacked in my garden.

2. Figure illustrating æcidium from Almora (by Dr. D. D. Cunningham).
3. Photograph of transverse section of leaf through an æcidium (photograph by Dr. D. D. Cunningham).

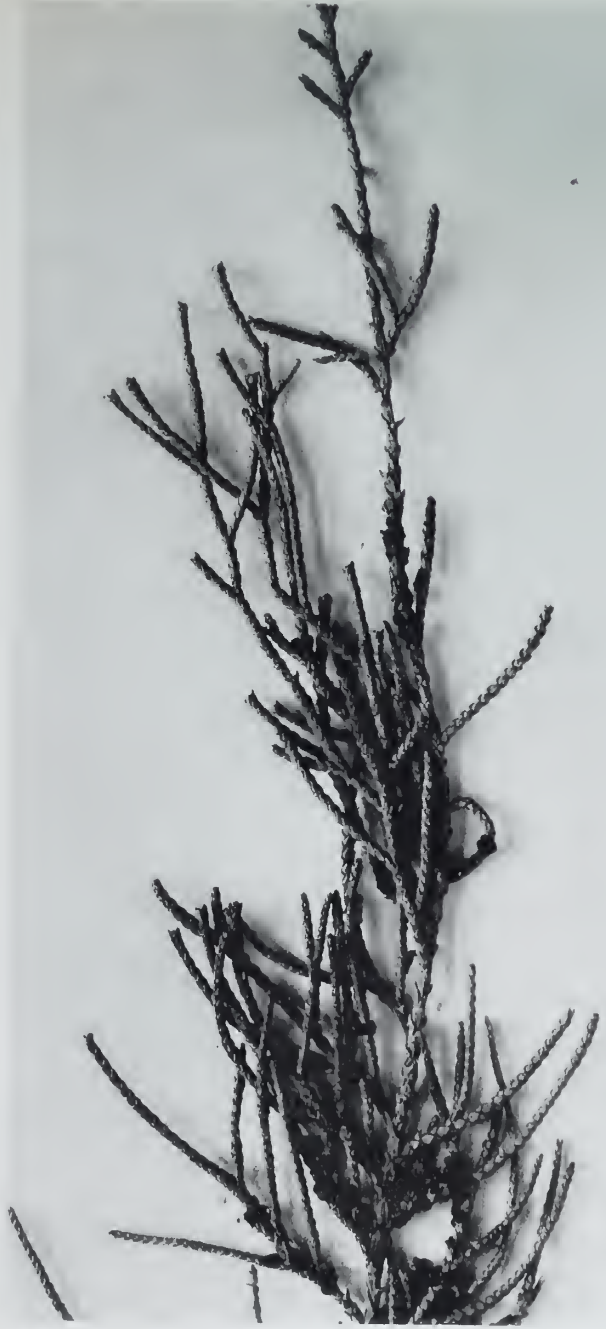


Fig 1

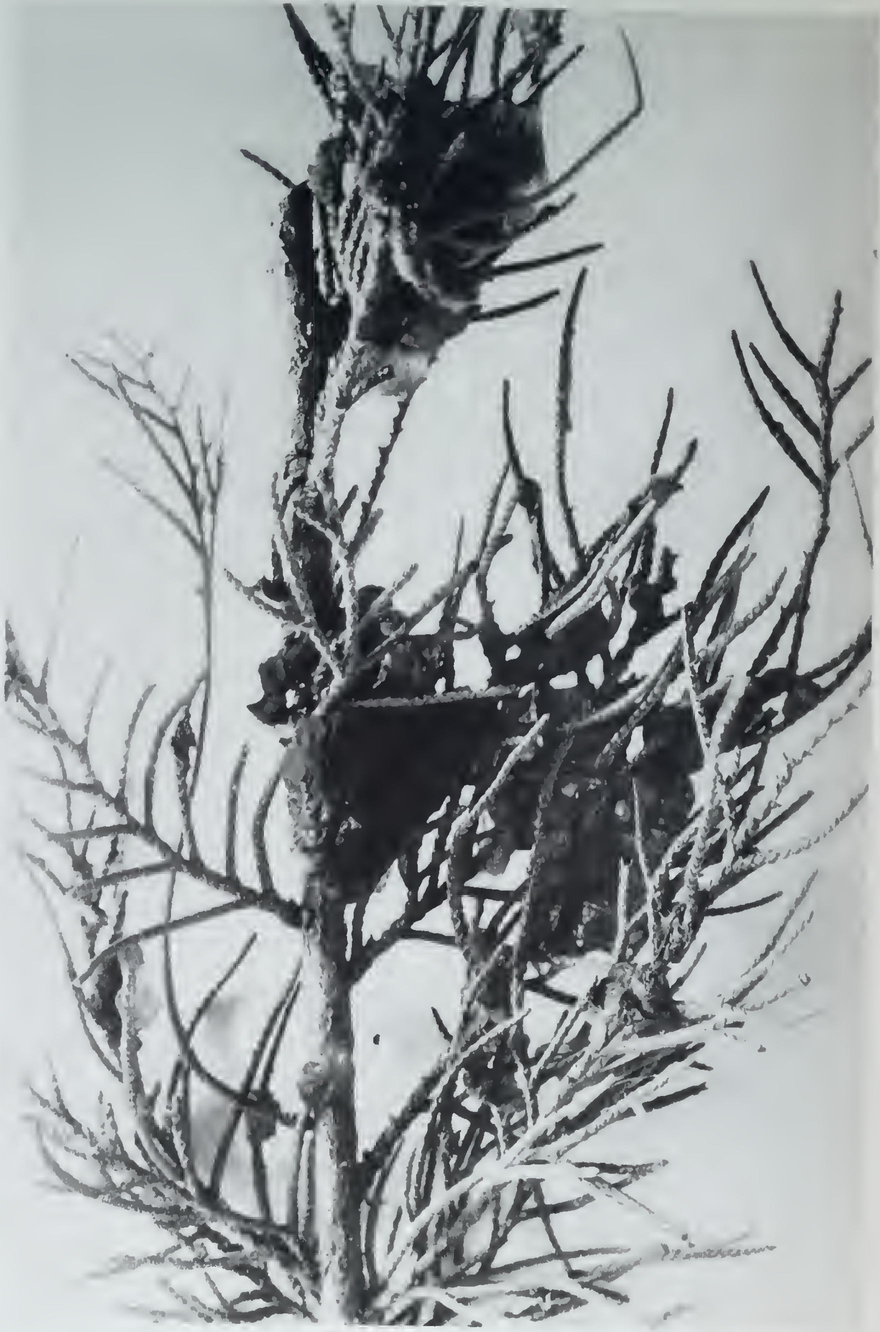


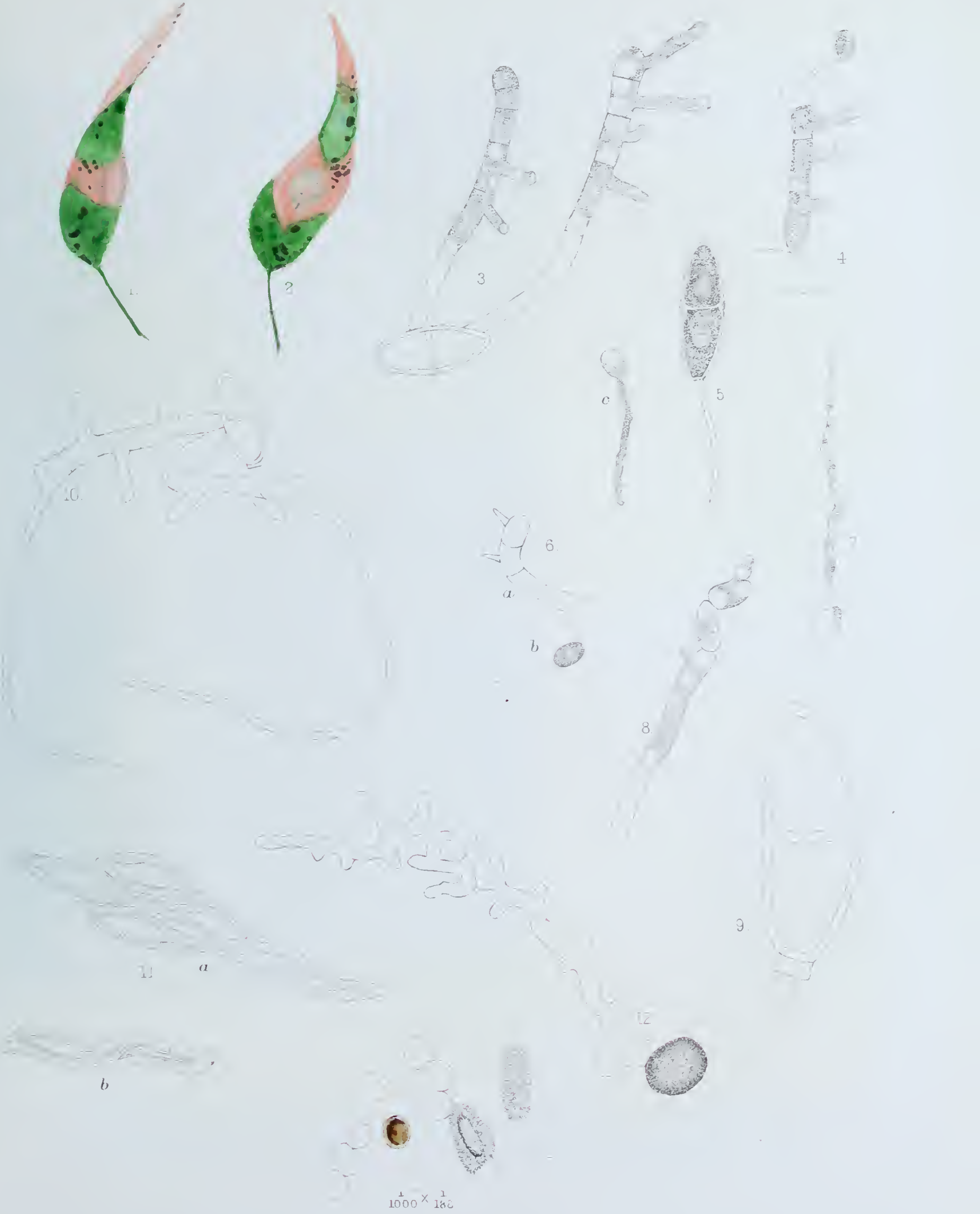
Fig 2

Photo-etching

Survey of India Offices Calcutta October 1889

GYMNOSPORANGIUM CUNNINGHAMIANUM

From photographs by A. Barclay



GYMNOSPORANGIUM CUNNINGHAMIANUM.

Litho., S. I. O., Calcutta.

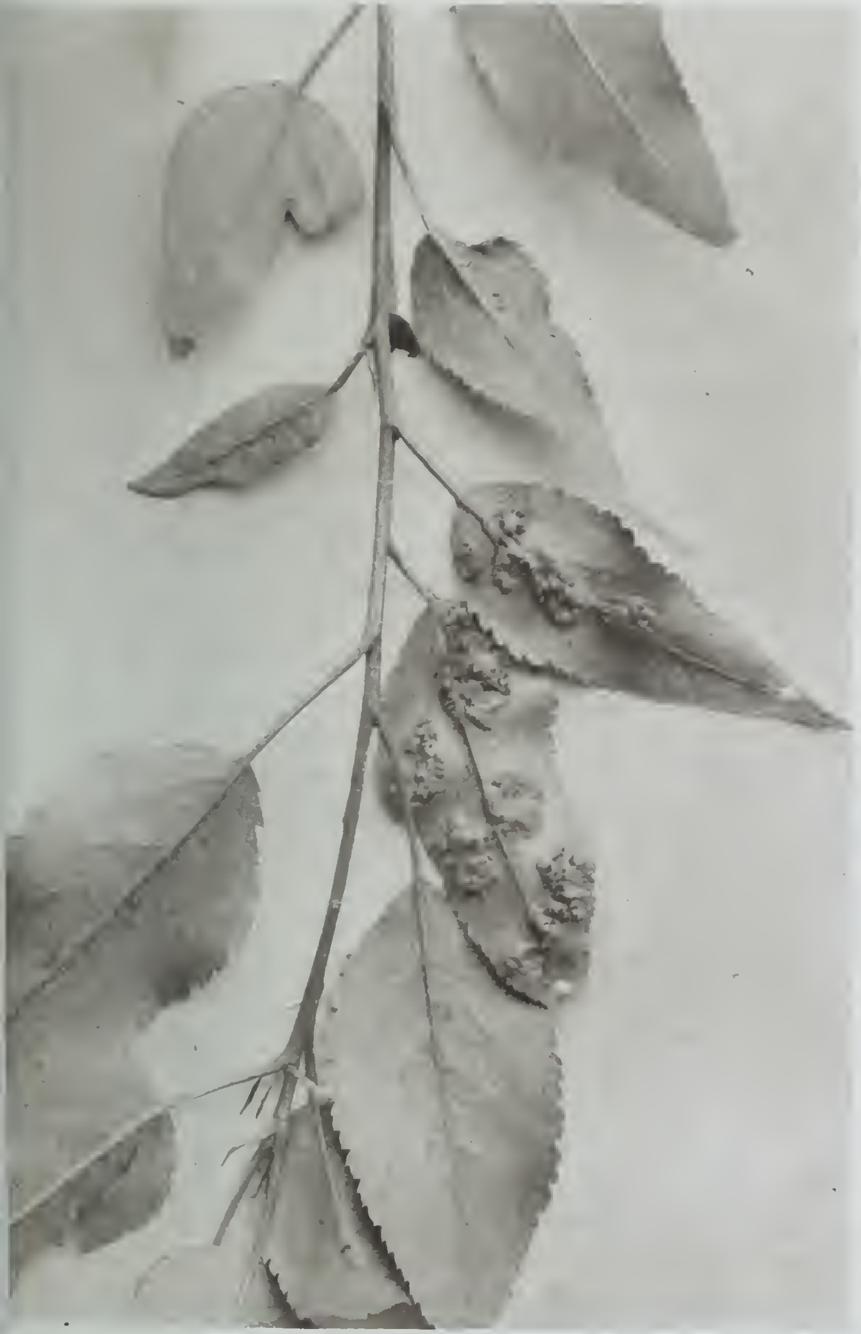


Fig 1

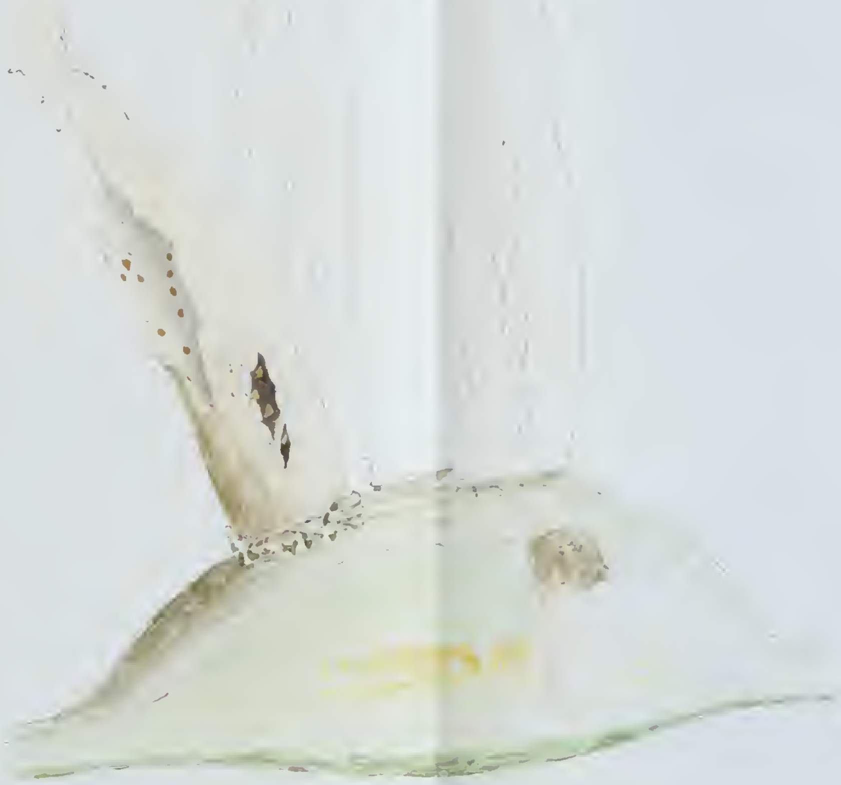


Fig 2

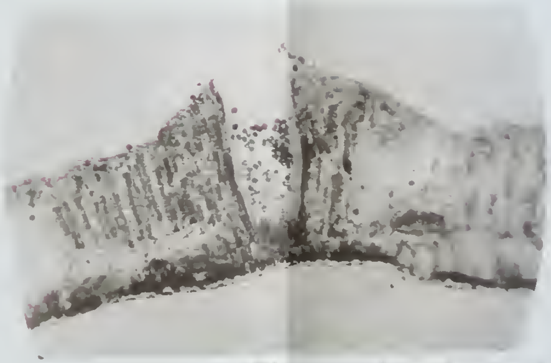


Fig 3

Photo-etching

Survey of India Offices, Calcutta, December [illegible]

GYMNOSPORANGIUM CUNNINGHAMIANUM.

A. Barclay

On a Chrysomyxa on Rhododendron arboreum, *Sm.*

(*CHRYSOMYXA HIMALENSE, NOV. SP.*)

BY

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This is an extremely conspicuous, and very widely distributed Uredine in the Simla region, where *Rhododendron arboreum* is most common. The mycelium is, for the most part, perennial, and attacked shoots are, year after year, so arrested in growth that a conspicuous clump of leaves ("witches' broom" or "Hexenbesen") is produced, by which attacked trees are readily distinguished at any time of the year. Were it not for this the presence of the parasite in any tree would be recognizable only from early spring to the end of May, during which time the remarkably striking fructification has commenced and ended. This has its seat especially on the petioles, and along the midrib a short distance into the leaf blade. When ripe, the fruit bodies clothe the petioles so densely as to hide it completely (Fig. 2, Plate I). Each separate fruit body is very prominently club-shaped, or mushroom-shaped. The expanded upper part measures on an average 2 m.m. in diameter, and the whole body about 1·5 m.m. in length from its base on the petiole. Some are, of course, a little larger and some smaller. But, although the fruit bodies are characteristically situated on the petioles and on the lower portions of the midribs, they are also occasionally found on the main axis of the shoot (Fig. 1, Plate 1), and as isolated groups on the leaf blade. During dry weather, or if they be kept a few hours in a moist atmosphere, they become pure yellow, due to rapid sporidial formation. This change in colour is very striking.

Very early in spring (March) the petioles of the leaves which were unfolded in the previous spring, but which are usually much smaller in size than normal leaves on healthy shoots, are seen to be uniformly orange red, while as yet there is no swelling or distortion of any kind. Although smaller, the leaves are not in any other respect different from normal leaves. Later, small wart-like projections are formed on the petioles, and these grow gradually until, towards the latter part of April and early May, they attain their maximum size and shape. While this maturation of the fruit bodies is proceeding, the Rhododendron trees are putting forth their new shoots and leaves, and the shoots which are invaded by the parasite, and on whose old leaves the fruit bodies are maturing, form no exception. The only difference is that, whilst normally the new shoots are elongated, and bear large leaves, those arising from an attacked shoot remain stunted, and put forth smaller leaves. Now, it is a significant fact that these newly unfolded leaves, whether on healthy shoots or not, although unfolded during the scattering of sporidia from the neighbouring fruit bodies of Chrysomyxa, never present any outward sign whatever of attack: they remain perfectly natural in appearance, though, as I have already observed, those arising from infected stems are usually smaller (Fig. 1, Plate I). This fact, of which I have convinced myself by repeated observation during the last three years, appears to render it extremely improbable that the fungus is autœcious, and it is not therefore comparable with *Chrysomyxa Abietis*. To this point I will, however, return. Another general point to which I may draw attention before proceeding to a detailed description of the fungus itself is that, although I have often searched for uredospores, I have never found any trace of them.

A.—Microscopic Characters of Fungus.

A.—On the Leaf-blade.—As already stated, localized affections of the leaf-blade are not common. When found, they are always on the older leaves of the preceding year's growth, and never on the newly unfolded leaves. In such cases there is usually but one group of fruit bodies (about 25 in number on an average) on the blade, distant from the midrib, and always on the lower surface. The position is indicated above on the upper surface by a reddish-brown patch of discolouration. The fruit bodies here are identical in shape and structure with those on the petiole, but are a little smaller.

The tissue of the leaf-blade is not much thickened at the sites of mycelial invasion. Whilst the whole depth of tissue in the blade of a normal leaf is about 0·315 m.m., at attacked places it is about 0·346 m.m. The mycelium is, however, very abundant in attacked areas, extending from midway between the inner of the two palisade layers of cells, which normally exist in these leaves, to the epidermis of the lower surface (Fig. 2, Plate II). The hyphæ, which measure on an average 5μ in diameter, are of the usual characters, but contain an abundance of orange-red oil globules; they ramify among the cells, sometimes destroying them, but never in this place penetrating them to form haustoria. The intercellular spaces in the spongy tissue are entirely filled up with mycelium. Indeed, in passing from natural tissue to the margin of attacked areas, the first noticeable variation consists in the obliteration of these air spaces, partly through their occupation by mycelium, and partly by cell proliferation (Fig. 3, Plate II). Immediately below the young fruit bodies which have just come to the surface, most of the spongy cells are disintegrated, but the palisade cells remain normal in appearance. The first commencement (inception) of the fruit bodies may readily be seen in leaves, and consists in the formation of a series of long septate upright cells, arranged at right angles to the surface of the leaf and in close contact with one another (Fig. 2, Plate II). These long cells measure about 14μ in diameter and 50 to 80μ in length. This initial body is formed in the middle of the leaf tissues, between the inner palisade row and the spongy cells, and is gradually pushed downwards by ever-increasing mycelial formation until it is ultimately pushed through the lower epidermis in its mature form.

B.—In the Petiole and lower Part of midrib.—It is here that the mycelium is seen in greatest profusion, for this is the usual site for the development of the fruit bodies. In transverse sections made through the middle of apetiole bearing ripe teleutospores, large aggregations of mycelium may be seen throughout the cortical parenchyma, occupying the large intercellular spaces which normally exist in this place, as well as in the stem (Fig. 6, Plate II). In addition to this, single hyphæ are, of course, also met with among the cells. This aggregation of mycelium is very characteristic, and comes prominently to notice, as the hyphæ everywhere contain an abundance of orange-red oil globules. There is apparently no destruction of cell tissue except at the bases of the fruit bodies, where again the mycelium is very abundant. The whole of the cortical parenchyma between the hypoderma and the central wood is thus invaded. In the earliest stages, when the fruit bodies are just being laid down (about four or five layers of cells below the epidermis), the central pith cells, surrounded by the crescent-shaped woody tissue, is not invaded, but later, as the horns of the crescent of woody tissue open out, the mycelium gains admission freely. In the tissue between the horns of the crescent, and just outside this, the mycelium is especially abundant. Haustoria, in the shape of simple straight, or slightly bent, short tubes are formed, especially in the cells of the central pith, and in the cells immediately outside the central wood bundle.

C.—In the Stem.—Mycelial filaments in the stem are found only in the cortical parenchyma, and never in the central pith. Such filaments may be seen even in the apparently as yet unattacked shoots put forth in spring from the axils of attacked leaves: for example, in the ultimate shoot shown in Fig. 1, Plate I. The mycelium can be seen in sparing amount in these new shoots at any time of the year. It is more abundant in the petioles of the dwarfed leaves borne by them, and even in August the cut surface of such a petiole is distinctly yellow to the naked eye from the presence of mycelium, although these petioles will not bear fruit bodies until the following spring.

D.—The Fruit body.—The structure of the fruit body, or sporophore, is very complicated in comparison with that of *C. Rhododendri.* In the first place, as will be seen in Fig. 5, Plate II, the body is a stalked mushroom-shaped structure. The diameter of the expanded head is almost 2 m.m.,; that of the stalk of the fruit body about 0·12 m.m., whilst the whole height is about 1·5 m.m., The cells composing the stalk of the body are long, narrow and septate. Higher up, in the expanded head, these primary stalk cells give rise to a series of large cells, three to four in number, which are usually branched or forked (Fig. 1 *b*, Plate II). The cells of this series, which practically make up the expanded head of the sporophore, are about 25×13μ, but vary considerably in size. These large cells again give rise, apparently successively, from above downwards, to narrow tubular prolongations towards the upper surface of the fruit body. These are about 80μ long and 4μ in diameter. When the end of this projection reaches the surface, it expands slightly, and then divides into four cells by transverse septa, forming a regular promycelium (Fig. 1 *a*, Plate II). Each cell of the promycelium then gives rise to a long narrow sterigma, bearing an oval or round sporidium at its end, orange red in colour, and measuring from 9×9 to 12×10μ. The sporidia are thrown off forcibly by some mechanism probably like that of Coprinus, just as they are said by de Bary to be thrown off in *C. Rhododendri.* The force by which this is accomplished is, however, feeble. Hence four sporidia are formed by each promycelium. Thus, four distinct parts may be distinguished in the sporophore, (*a*) the primary lowermost stalk cells, (*b*) the large central cells which give rise to the promycelia, (*c*) the secondary stalk cells, of the promycelia, and (*d*) the promycelia proper, with sterigmata and sporidia. The sporidia germinate at once, throwing out the usual germ tube, and this may give rise to a secondary sporidium (Fig. 4, Plate II). The promycelia measure about 50μ in length by 10μ in breadth.

B.—General Remarks.

Having thus concluded a description of this parasite, I will pass on to more speculative ground and consider the relation of this Himalayan form to the European species, and to its possible connection with the æcidial forms which occur on the Coniferæ in this region, for, despite many and prolonged attempts to discover what becomes of the sporidia so abundantly shed every year, I have never succeeded in following its history. From the very clear description of the European species given by de Bary,[1] it would appear that this Himalayan species differs considerably from *Chrysomyxa Rhododendri* (D.C.) The most striking difference consists in the truly perennial habit of the mycelium in the Himalayan species, and in the usual seat of the eruption of spore beds, two characters which give it a very peculiar appearance. The mycelium of *C. Rhododendri* is mentioned as occurring only in localized areas of the leaf-blade, and as disappearing therefore with the fall of the annual leaves bearing them. In this respect there is as much difference between these two species as exists between the Æcidium of *P. Graminis* and *Æcidium Magelhaenicum,* Berk, on the Barberry. Another important difference consists in the absence of uredospores in the Himalayan species,

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[1] *Æcidium abietinum,* Botanische Zeitung, Nos. 48 to 52, 1879.

I have searched in vain for such spores, both at elevations below and above that of Simla. A third important difference may be found in the structure of the fruit bodies, and in the way in which sporidia are formed. A comparison of Fig. 5, Plate II, representing a section through a teleutospore bed, with de Bary's figure, as reproduced, for example, in his-text book,[1] will at once reveal the striking differences. Among subsidiary points of difference may be mentioned the form of the teleutospore beds, which are sometimes long in *C. Rhododendri*, but always round in the Himalayan species: the larger size of many of the beds in the latter: the golden red colour of the teleutospore beds in sporidial formation in *C. Rhododendri* against a pure yellow in India: the almost invariable division of the promycelium into four cells in the Himalayan species, each cell forming an oval or round sporidium at the end of a long sterigma. These differences appear to me to be sufficiently great to warrant its separation under a new name, and I propose calling it *Chrysomyxa Himalense.*

I will now offer a few remarks with reference to its possible connection with the æcidia on the Coniferæ occurring here. Of these there are four, namely, one on *Pinus longifolia*, one on *Pinus excelsa* (these two are, I believe, specifically distinct), and two distinct species on *Picea Morinda.*[2] The two first named are exceedingly common in Simla; one of those on Picea is rare, whilst the other does not occur in Simla itself, though it is not uncommon at some little distance from the station. It seemed extremely probable that one or other of the æcidia on the two species of Pinus, so abundant in the forests where the Rhododendron is largely and universally attacked with this Chrysomyxa, must be related to it, although I am aware a different life-history has been discovered for *Peridermium Pini* in Europe. I refer, of course, to the connection with *Coleosporium Senecionis*, Per (Wolff) and *Cronartium asclepiadeum* (Willd.) on *Vincetoxicum officinale* (Cornu). Neither of these teleutospore forms occurs in Simla, nor indeed any allied form, so far as my observations as yet extend.[3] If the Indian Æcidia in question are heterœcious, as there is every reason for believing they are, their teleutospores must be looked for elsewhere. I accordingly made frequent attempts to establish a relationship with this Chrysomyxa, but all my efforts have been in vain. I never obtained any evidence of connection. I also applied sporidia to the young shoots of *Picea Morinda* with equally negative results. The last-mentioned host is not common in Simla, but the few specimens that occur are surrounded in every direction by Rhododendron trees bearing the Chrysomyxa, so that if a connection existed the æcidium on Picea must have been a common instead of a very rare parasite. It is scarcely possible, therefore, that the last-mentioned Æcidium can be related to the

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[1] Comparative Morphology and Biology of the Fungi: De Bary, Clarendon Press, page 284.

[2] Journal of Asiatic Society of Bengal, Vol. LV, Part II, Nos. 1 and 2, 1886,

[3] We have several species of Coleosporium, however, in Simla, but none of them with any apparent connection.

Chrysomyxa. But, although my attempts to reproduce either Æcidium on the two species of Pinus have failed, I am not so confident that one or other may not be caused by the Chrysomyxa, because their abundance runs parallel with the abundance of the Rhododendron affection. Again, if I am correct in regarding the two Æcidia on the two species of Pinus as distinct, then, for the following reasons, I think that on *P. excelsa* is more likely to be the one so related.

The natural history of the *P. longifolia* Æcidium runs thus:—A few ripe æcidia may be found on the needles as early as January and February, but they become more and more abundant as time goes on, until in May it is exceedingly abundant. But at this time the needles which bear them, and which were formed in the *preceding* spring, are rapidly falling off, and in June defoliation is complete. While this is going on new needles are being unfolded, commencing in March, and by the time the old needles bearing the æcidia have all fallen, the new ones are about one-half to three-quarters grown. These new needles present no sign whatever of attack. The host, therefore, remains free of the fungus until very early next spring, when the whole cycle of events above described is repeated. Thus the æcidia begin to appear long before the Chrysomyxa is ripe and continue to be formed, while the Chrysomyxa sporidia are being shed, but only on the older needles. Hence if these sporidia give rise to the æcidia, attack of the new needles must take place during April and May, although no outward sign of it exists, and then a long peroid of latency of the mycelium ensues until early next spring. This, however, appears to me to be very improbable.[1]

The natural history of the *Pinus excelsa* Æcidium is much the same as the above, but with these important differences. The æcidia do not appear until April and May, when Chrysomyxa sporidia are beginning to be scattered, and the needles are not shed annually. But here also only the needles unfolded in the previous year are attacked, and never those formed at this time. In this case, therefore, the season of the appearance of the æcidia makes it quite possible that it is caused by the Chrysomyxa sporidia: both fungi appear and ripen simultaneously. In this case we do not need to assume initial attack, with a subsequent period of quiescence. However, as I have already noted, I have not been able experimentally to produce attack in the needles with Chrysomyxa sporidia.

Conversely, I have often also tried to reproduce the Chrysomyxa on Rhododendron by laying the æcidiospores from both species of Pinus on the leaves, but with no success.

Thus, though there is presumptive evidence to favour a view of connection between the Æcidium on *P. excelsa* and *Chrysomyxa Himalense*, experimental evidence, so far, is against it. If the connection does not in fact exist, I am quite at a loss to guess what other host the sporidia attack. I have already given reasons for excluding the idea that the fungus is autœcious. On the one hand, it is probable that so widely diffused a teleutosporic form is connected with a widely distributed Æcidium, but, on the other hand, considering the advantage gained by the teleutosporic form in having a resistant perennial mycelium, it is quite possible that it may be connected with a very rare Æcidium ; and that immense numbers of sporidia are formed to ensure an occasional completion of life-history. With this possibility in view I have applied the sporidia to several hosts bearing æcidia in this neighbourhood, but always without result.

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[1] As this paper was passing through the press I found that some few isolated fully ripe æcidia may be found in autumn.

With regard to the geographical distribution of this parasite I have no information. From some information very kindly given me by Mr. Gamble, Conservator of Forests, Madras, I infer the possible existence of a Chrysomyxa at Ootacamund in the Nilghiris on *R. arboreum*, var. *nilagirica*. Some fungus on this tree produces clumps of "witches' broom," and in all probability this is a Chrysomyxa ; but I have not yet been able to examine it. There are no native Coniferæ in this place, and Mr. Gamble has not looked for any æcidia on the cultivated trees. He has, however, kindly promised to look for any such in the coming season.

Description of the Plates.

PLATE I.—1. Photograph, natural size, of a stunted shoot, showing the stem and petioles of the older leaves covered with fruit-bodies, and the younger leaves, just unfolded, quite free.

2. Photograph, natural size, showing two separated older leaves with petioles covered with fruit-bodies.

PLATE II.—1. Promycelia: (*a*) × 350, showing promycelium divided into four cells, each with a sterigma from which the sporidia have fallen off: (*b*) × 150. showing two promycelia, with their stalks and the central cells giving rise to them.

2. Transverse section of leaf showing inception of fruit-bodies, × 150.

3. Ditto at margin of area of invasion. At (*a*) the intercellular spaces are filled with mycelium: at (*b*) the tissue is normal, × 150.

4. Sporidia showing germination, and formation of secondary sporidia, × 580.

5. Photograph of transverse section of petiole, by Dr. D. D. Cunningham, showing mature fruit-body (compare with DeBary's figure of *C. Rhododendri*).

6. Photograph of cortical tissue of petiole by Dr. D. D. Cunningham, showing aggregations of mycelium in intercellular spaces.

PLATE

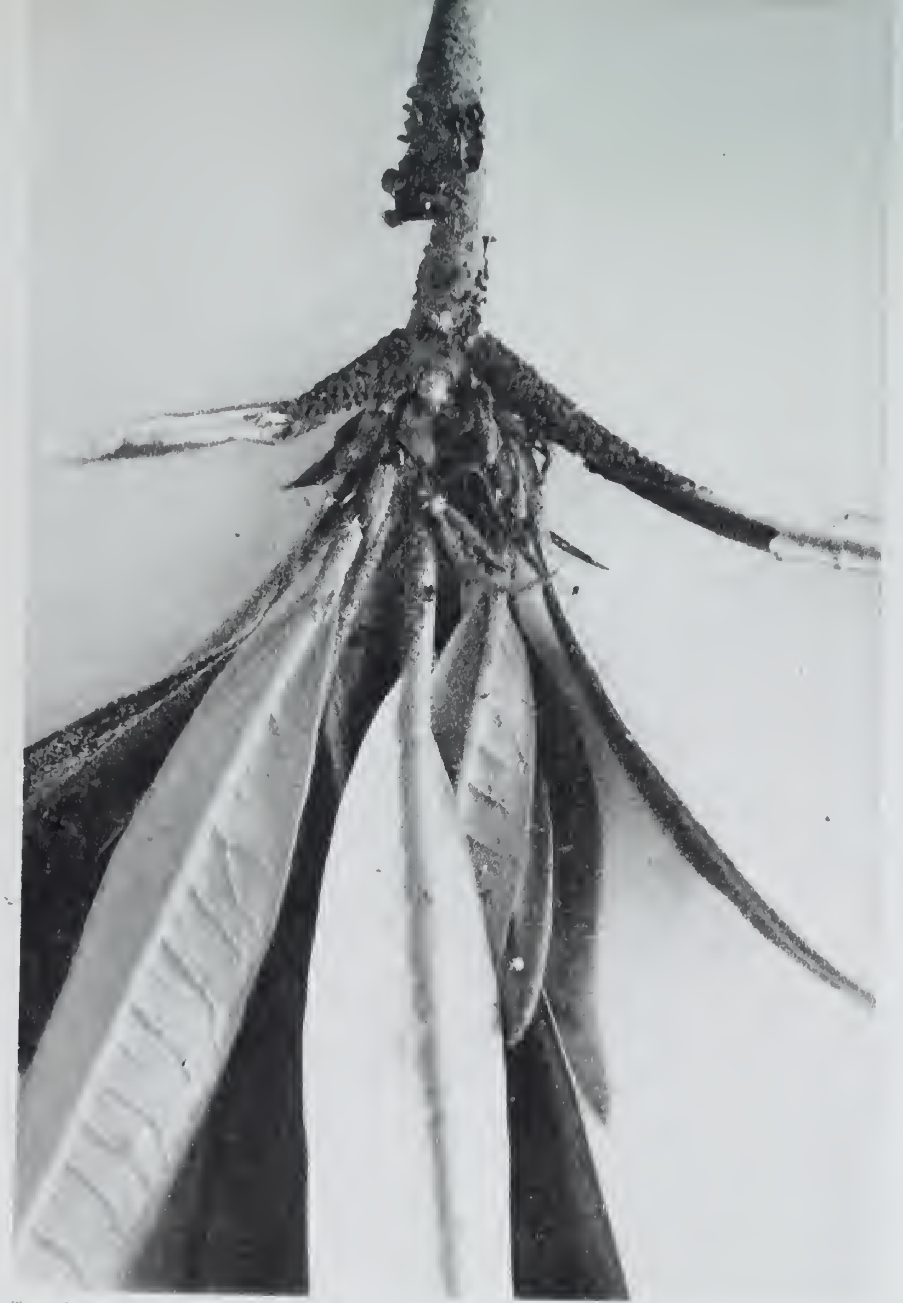


Fig 1

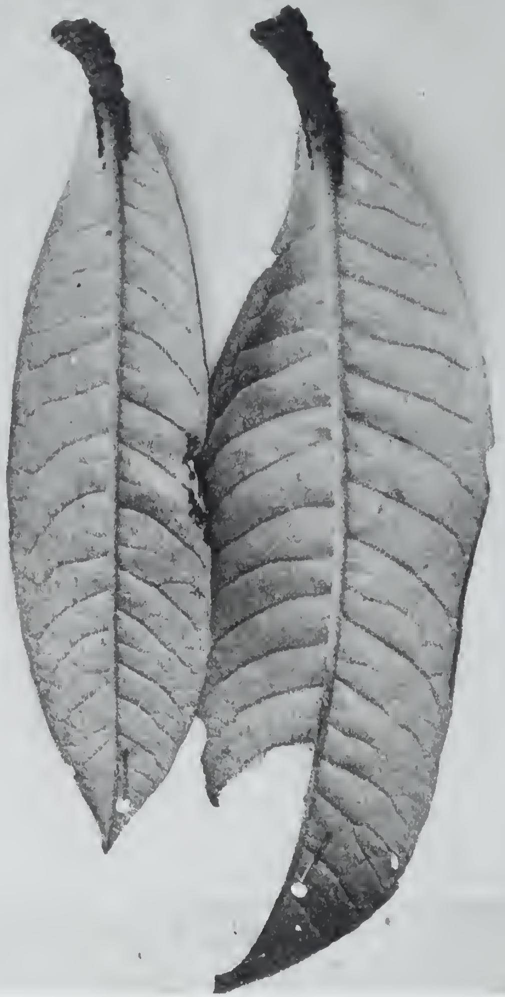


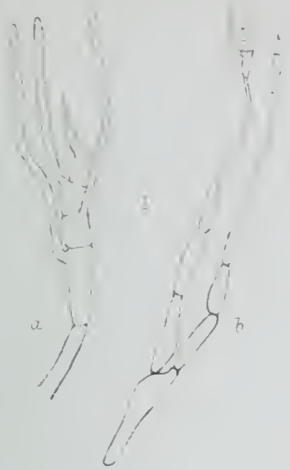
Fig 3

CHRYSOMYXA HIMALENSE

From Photographs by A.Barclay

Photo-etching

Survey of India Offices.Calcutta November 1883



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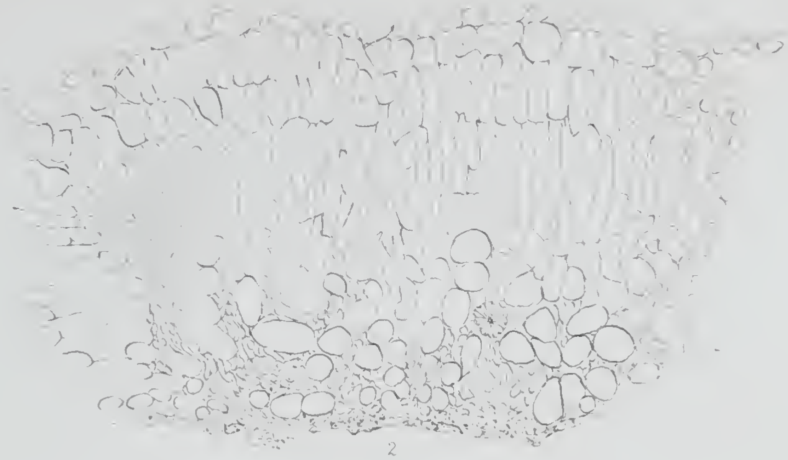

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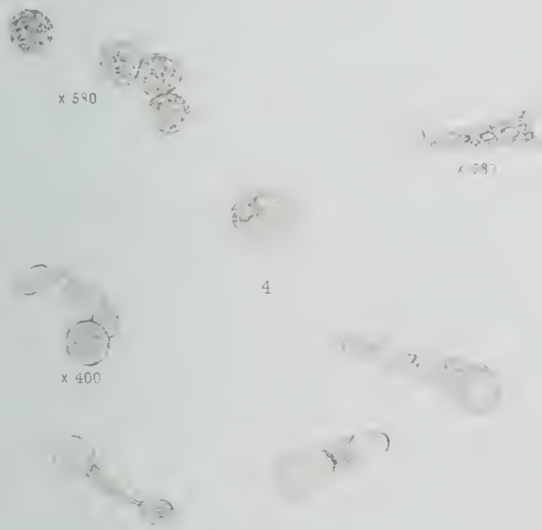

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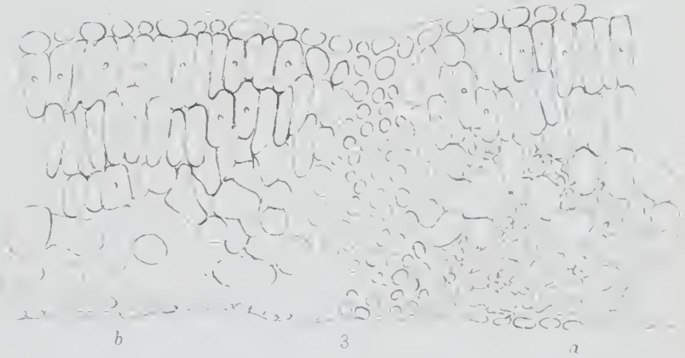

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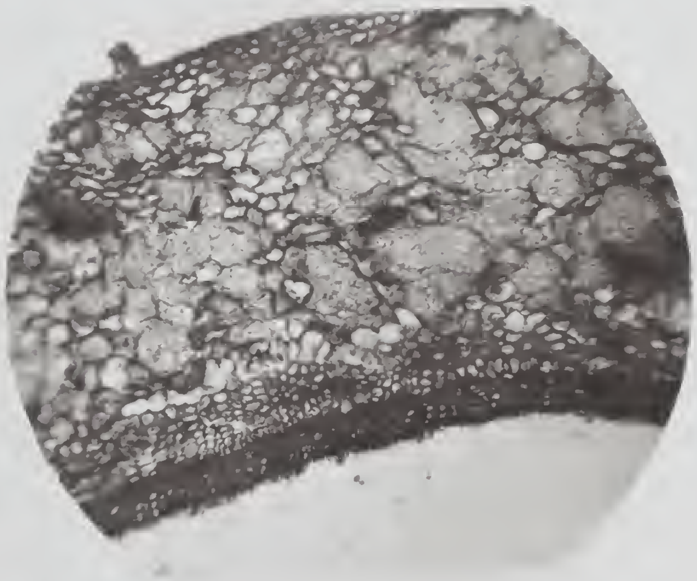

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Photo-etching

CHRYSOMYXA HIMALENSE

A. Barclay

Survey of India Offices Calcutta January 1890

On the Life-history of a Uredine on Rubia cordifolia, Linn.

(*Puccinia Collettiana, nov. sp.*)

BY

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In this species we have an interesting, and experimentally proved example of the complete exclusion of æcidial fructification. The natural history of the fungus led me long ago to believe that it was a complete autœcious species; but, in the absence of experimental evidence, I was obliged to disregard my conviction, and in my descriptive list of the Uredineæ of the Simla region,[1] I named it provisionally *P. helvetica*, Schröter, noting my hesitation whether it was correctly so named. Since then I have found by experiment that the species is, indeed, complete without any æcidial fructification; and it must, therefore, be a new species, as I can find no evidence that Schröter's *P. helvetica* is a complete species.

Although a few new shoots and leaves may be found on this host in certain favourable localities as early as April, yet these are never found attacked in nature. The host is not really in full vegetative activity until moist weather sets in, from July to October, and it is then that it is attacked by this fungus. Thereafter the plant dries up, and the persistent leaves remain for the most part attached to the apparently dry stems throughout the winter, and until the next period of vegetative activity recurs. These dried leaves may often be seen bearing immense numbers of round black Puccinia pustules, frequently with a markedly circinate arrangement, both on the upper and on the lower surface of the leaf, but mostly on the upper. The petioles and stems also bear them, the pustules here being usually elongated. The teleutospores from these pustules do not germinate at once, but only after a period of rest, *i e.* not until the following spring. In nature they do not, as a rule, germinate until July, but I have observed them germinating in my laboratory as early as April. This they do in the usual manner (Figs. 2 and 6, Plate I). The promycelia divide into four strikingly regular cells, and each produces a sporidium at the end of a long finger-like sterigma (Fig. 2, Plate I). The sporidia are oval, measuring from 12·5 × 10μ to 14 × 8μ. They germinate at once upon becoming detached, in the usual way (Fig. 5, Plate I).

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[1] Journal of the Asiatic Society of Bengal, 1889, Vol. LVIII, Part II.

[2] Coloured illustrations of the natural appearance of the fungus are given in the Journal of the Asiatic Society of Bengal above referred to.

When the host bears young leaves the sporidia attack them, as well as the green petioles and stems, producing slightly paled areas, on which groups of very pale spermogonia are developed, very inconspicuous to the naked eye. Soon after this, primary uredopustules are produced, immediately outside the group of spermogonia. On the leaf-blade these primary pustules generally assume a markedly circinate arrangement. Both the spermogonia and the primary uredopustules are produced on both surfaces of the leaf blade, most numerously however on the lower surface. The pustules on either surface of the leaf-blade are often exactly opposite one another. On leaf-blades immense numbers of such pustules are usually formed. Each separate area of invasion is usually about 3 m.m. in diameter; but such areas frequently coalesce. The uredospores contained in these pustules germinate, as usual immediately, and distribute the fungus largely. A straight germ tube, about 6μ in diameter, is usually formed, into the end of which the colourless granular contents wander (Fig. 1, Plate I).

These uredospores produce vast numbers of secondary uredopustules, without, however, any foregoing spermogonia. These secondary pustules do not, as a rule, assume the circinate arrangement so common in the case of the primary pustules. They are, indeed, usually isolated scattered minute brown pustules, also on both surfaces of the leaf, and also mainly on the lower surface. These uredospores are morphologically identical with the primary uredospores.

As the vegetative period of the host approaches an end, immense numbers of teleutospore pustules are formed, thus completing the whole cycle of development. The same mycelium which produced secondary uredospores produces these teleutospores, but mostly apparently in distinct pustules. At any rate, from the fact that the teleutospore pustules are usually borne on the upper leaf surface, whilst the uredopustules are mainly borne on the lower surface, the great majority of the former must be distinct in origin from the latter. I have, however, found a few uredospores in teleutospore pustules, and some teleutospores in secondary uredopustules.

There is no vestige of æcidial fructification.

With regard to the experimental evidence that the above life-history is complete, I will give the details of some experiments I made with this view. On the 24th April, I placed some sporidia, obtained by floating scraped off teleutospores on water in watch-glass (kept in a moist atmosphere to prevent evaporation) on the leaves of a twig cut off under water, and kept in my laboratory under a glass shade. On the 9th May (15 days later) I noticed a large patch of paled tissue on one leaf, with several spermogonia; but on the 21st May many leaves bore spermogonial patches on numerous paled areas. Again, on the 25th June, two plants were similarly inoculated with sporidia: they were then placed in a closed glass gardener's box outside; one (a) was again covered there by a glass shade, and the other (b) was left uncovered. On the 4th July, 9 days later, I observed very numerous points of attack on many leaves of each plant. On the

10th July, the plant (*a*) displayed 25 leaves covered with spermogonial patches, whilst plant (*b*), though also very largely attacked, was not so greatly involved as plant (*a*). The latter was in a moister atmosphere, which must account for the slight difference. These plants produced later an abundant crop of uredospores, and on the 25th July, exactly a month after the primary inoculation, I took off some uredospores, and, placing them in water, found that they germinated freely and normally (Fig. 1, Plate I).

I then made inoculation experiments with primary uredospores on healthy leaves. For example, on the 22nd August, I placed some uredospores upon some drops of water which I had previously placed on young healthy leaves. I then placed the inoculated plants (which were cut twigs with their ends in water) in a glass box outside, within which the air is always very moist. On the 2nd September, I noticed several pustules just breaking through the epidermis, and although I searched both then and later carefully for spermogonia, I could find no trace of them. Spermogonia appear therefore to be formed only by the mycelia produced by sporidia, and not by the mycelia produced by uredospores.

Unfortunately all my experiments were made upon cut twigs, and none of them survived long enough to produce teleutospores. But the evidence already given is quite sufficient to establish the fact that the species is complete, as I have described it. I marked numerous leaves in nature in the secondary uredo stage, and found them bearing teleutospores towards the end of August.

This fungus would come under Schröter's group Brachypuccinia, and is allied to such forms as *P. suaveolens* (Pers.), *P. Oreoselini* (Strauss), *P. Vincæ* (D. C.), *P. fusca* (Relhan), *P. Taraxaci* (Plow.), *P. bullata* (Pers.), *P. Hieracii* (Schum.), and *P. Centaureæ*, (Mart.). In *P. suaveolens* and *P. fusca* the mycelium is perennial, or at any rate not localised. In *P. Vincæ* spermogonia are sometimes formed, and sometimes not. In *P. fusca* spermogonia accompany the teleutospores, and æcidial fructification is rare in England according to Plowright.[1] In *P. Taraxaci* spermogonia and uredo pustules are first formed, as in the species under description, and teleutospore pustules later: and, while Plowright states that he has never seen any associated Aecidium, Grove asserts that it does occur.[2]

This species would appear to lend strong support to the view now generally gaining ground, especially on the authority of Brefeld, that spermogonia are not sexual organs, but that the so-called spermatia are a form of conidia, like sporidia. The only explanation that can be given in a case like the present in favour of the older view is, either that the spermogonia are in course of being suppressed, as in the case of *P. Vincæ*, or that, although the æcidial fructification is usually dropped out of the cycle of development, it may occasionally re-appear, and that on the assumption that this fructification is a sexually developed one, the male organs are retained for occasional sexual reproduction, as in *P. fusca*, and perhaps also in *P. Taraxaci*. There is, however, no sign of immaturity as yet in the development of spermogonia; on the contrary, they form a prominently characteristic feature; and during the four years I have watched the fungus, I have never seen an occasional æcidium. On the other hand, this fungus also negatives the view that the spermatia may exercise a sexual function in the production of teleutospores, as most, if not all, of these are formed by mycelia produced by primary uredospores, which bear no spermogonia, and even the teleutospores that may be borne by mycelia producing spermatia are formed long after the spermogonia have withered. (I must confess that I only assume that the mycelia produced by sporidia bear teleutospores after a sufficient number of uredospores have been shed; I have no direct evidence that this is actually the case, as I unfortunately did not think of the matter in time. My impression is that teleutospores are not borne by mycelia bearing uredospores and spermogonia.) Lastly, it is obvious also that the spermatia have no causal connection with the production of uredospores. The secondary uredospores are morphologically undistinguishable from the primary, and the latter alone are accompanied by spermogonia.

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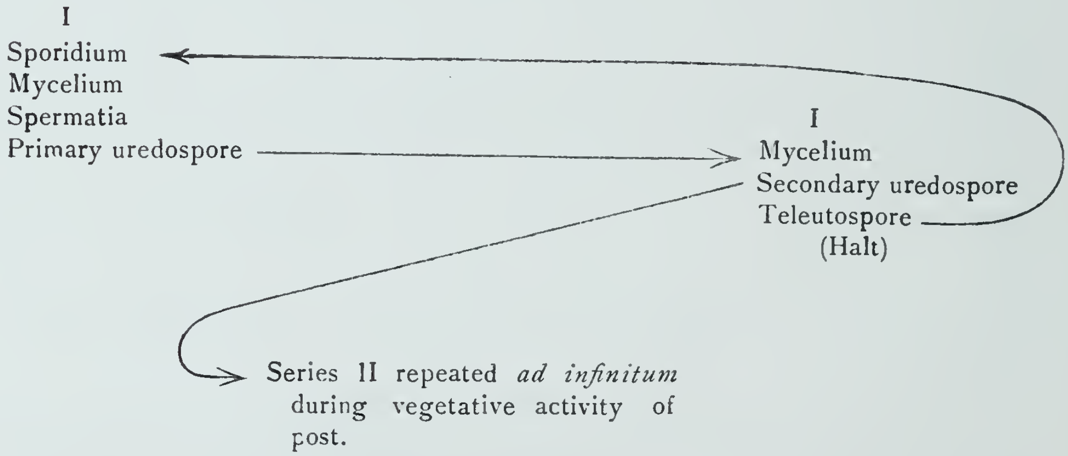
[1] British Uredineæ and Ustilagineæ, p. 182.

[2] Plowright, *loc. cit.*, p 187.

The sporidium differs from the uredospore physiologically in producing a mycelium, which bears spermatia as well as uredospores.

The cycles of development may be shown graphically thus—



The diagnostic characters of this fungus, which I propose naming Puccinia Collettiana, after my friend Colonel Collett, who is as accomplished a botanist as he is a distinguished soldier, are briefly as follows:—

SPERMOGONIA: appearing earliest on mycelia produced by sporidia; mainly hypophyllous; a tuft of paraphyses protruding through the mouth; spermatia round to oval, 3μ in diameter to 6×2 or 3μ.

UREDOSPORES: (*a*) the *primary* on mycelia produced by sporidia; pustules mainly hypophyllous, mostly circinate: (*b*) the *secondary* on mycelia produced by uredospores; pustules mostly hypophyllous, not circinate. Both brown, beset with prominent spines, not however very closely aggregated; round to oval, varying considerably in size, from 22μ in diameter to 30×21 to 33× 25μ; on an average 27×22μ; each spore with two germ pores.

TEELEUTOSPORES: in pustules often circinate; mainly epiphyllous; dark brown, two-celled (sometimes an occasional three-celled spore may be seen as represented in Fig. 4, Plate I); constricted at the septum; well marked apical thickening; surface smooth; total length 49 to 54μ by 13 to 15μ at the septum; sporidia oval, 12·5×10μ to 14×8μ.

ÆCIDIOSPORES: absent.

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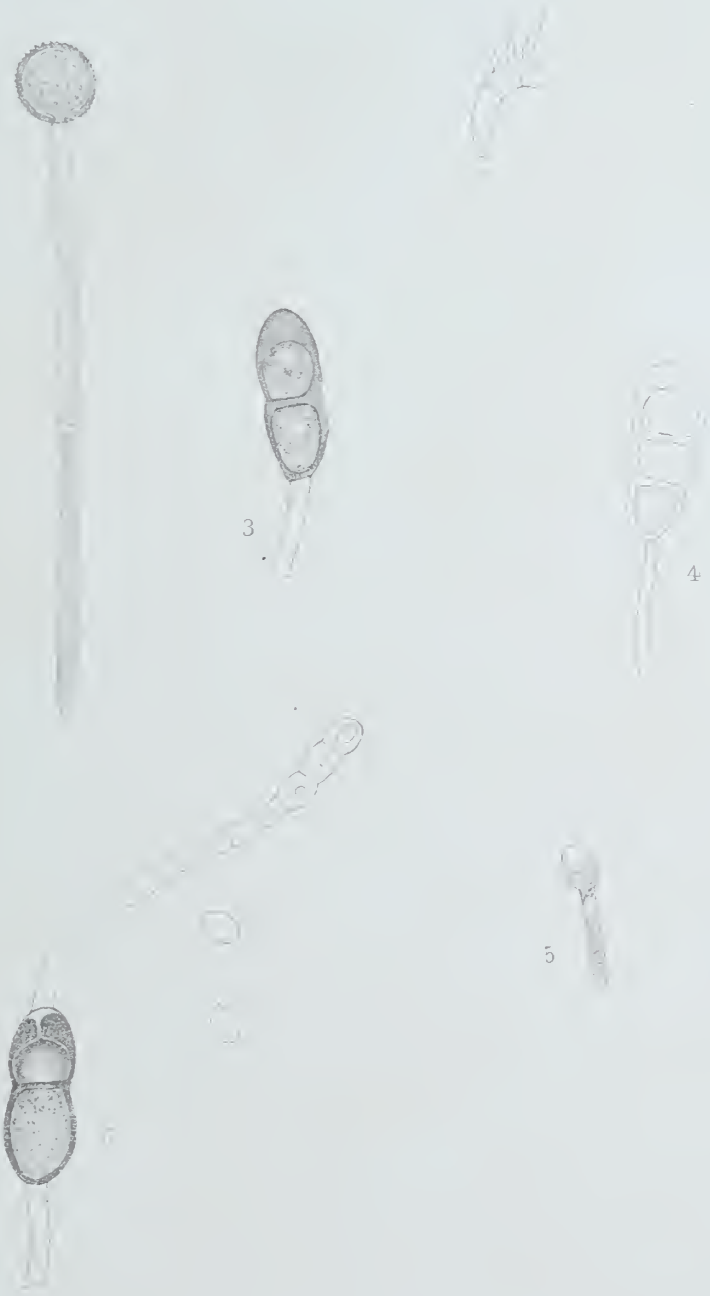
Description of the Plate.

1. Primary uredospore germinating, × 350.
2. Upper end of promycelium, × 350.
3. Typical teleutospore, × 350.
4. Three-celled teleutospore in outline, × 350.
5. Germinating sporidium, × 350.
6. Germinating teleutospore showing curiously regular septation, × 350.



A. Barclay.

PUCCINIA COLLETTIANA.

No 2692, San —27-1-89 —350.

Litho., S. I. O., Calcutta

SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

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SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

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